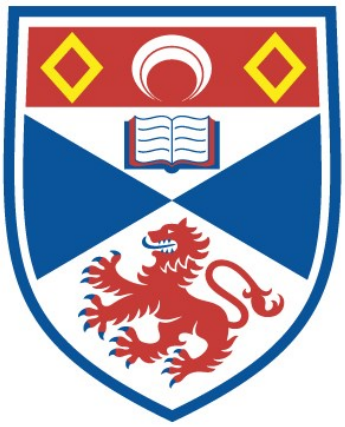
ELECTROPHYSIOLOGICAL STUDIES ON THE OPTICS OF THE COMPOUND EYE

John Tunstall

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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by

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and
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SUPERVISOR'S CERTIFICATE

I certify that John Tunstall has fulfilled the conditions laid down under Ordinance No. 16 of the University Court, St. Andrews, and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

DECLARATION

I declare that the work reported in this thesis is my own and has not been previously submitted for any other degree.

VITAE

I was educated at Carlisle Grammar School and at King's College, University of Durham where I graduated in Zoology in 1962. I came to St Andrews in October of that year. The early part of my Ph.D. period was spent on work concerned with nervous integration in the central nervous system of insects. The work reported here was done in the years 1964 - 1966.

CONTENTS

3. RESULTS.

4. DISCUSSION.

ABSTRACT

1. Intracellular recordings from single retinula cells in the locust compound eye show that the visual field of a single cell is wider in dark-adapted than in light-adapted eyes.

2. The time course of the increase in the visual field of a single cell is correlated with the replacement of a dense packing of mitochondria by a fluid filled palisade around the rhabdom during dark-adaptation.

3. The development of the palisade results in a reduction of the refractive index immediately around the rhabdom.

4. The correlation between the increase in the visual field of a single cell and the development of the palisade is evidence for internal reflection in the rhabdom.

5. Direct tests of the acuity of single retinula cells to equal black and white stripes of various repeat distances show reasonable agreement with theoretical considerations based upon Gaussian acceptance curves, and is dependent upon the state of adaptation of the eye.

1. INTRODUCTION

The unique feature of the arthropod compound eye is its reduplicated optical system. Since the axes of the units in the optical system diverge, each surveys a slightly different part of the total visual field of the eye, and transfers its part of the field to the underlying cluster of photoreceptor cells.

A lens or dioptric system with its associated photoreceptor and pigment cells comprise the ommatidium, the fundamental structural unit of the compound eye typically repeated many times in the whole eye. The ommatidium is a functional unit as well as a structural unit, but the extent of its independence within the compound eye as a whole and its nervous connections are poorly understood.

Because of the divergence of the ommatidial axes, the directional sensitivity of a single unit in the compound eye will be one of its fundamental physiological properties. Similarly the directional sensitivity of each unit in the eye will determine the optical and physiological properties of the whole eye.

The classical theory of insect vision, the mosaic theory (Müller 1826; Exner 1891) assumes the ommatidium to be the basic functional unit of the compound eye with a visual field restricted to axial or near-axial rays (See section A2). This theory, which has now been shown to be wrong in many details, rationalized differences in structure and pigment motility in the apposition and superposition types of eye and related them to the behaviour of various arthropods.

At the present time there are two quite distinct theories of the optics of compound eyes. Both agree, however, that light enters the ommatidium from an angle in the environment which is greater than the geometrical projection of the ommatidium.

According to one theory (de Vries and Kuiper 1958; Kuiper 1962), the rhabdom which has a higher refractive index than the cytoplasmic contents of the surrounding retinula cells, acts as a wave-guide and channels an incident cone of light rays into the deeper parts of the retina. On this theory the angular extent of the ommatidial visual field is determined by the critical angle for internal reflection at the rhabdom/cytoplasm interface.

The second theory (Burtt and Catton 1961 a,b; 1962 a,b), in contrast, presumes that light rays passing into the ommatidium are not contained in the rhabdom by reflection at the rhabdom/cytoplasm boundary, and are able to pass into adjacent ommatidia. A type of super-position image is then formed by the diffraction optics of several corneal apertures.

As both of these processes can be directly observed in sections of retinal tissue, the most important one physiologically is likely to be that which brings the most light to the photosensitive regions. It still remains to be shown, however, how sub-retinal nervous interaction may compensate for mosaic blurring that must result from the wave-guide activity of the rhabdoms on one theory, or make use of the magnified diffraction images at various levels in the eye on the

other theory.

During dark adaptation in the locust compound eye, the refractive relationship between the rhabdoms and surrounding retinula cytoplasm is altered (Barnard (1964) Horridge and Barnard (1965)). On the wave-guide theory of the rhabdom such a change, by altering the critical angle for internal reflection, should have an effect upon the ommatidial acceptance angle.

This thesis is concerned with the directional sensitivity of both dark-and light-adapted retinula cells in the compound eye of the locust (Locusta migratoria), its relationship to the interommatidial angle, and to what extent it is limited by internal reflection in the rhabdom.

Also, the ability of both light- and dark-adapted retinula cells to resolve the motion of striped patterns is tested directly. The ability of the cells to resolve pattern motion is related to their directional sensitivity and is compared with theoretical curves of contrast transfer (Götz 1964; 1965).

Finally, the results have some bearing upon the ability of light rays to spread into neighbouring ommatidia, which is a basic requirement for the diffraction theory of superposed images from different ommatidia.

A. Background to the Thesis

1. Anatomy

Since Exner (1891), two distinct types of compound eye are recognised.

i) Apposition eyes characteristic of most diurnal insects are presumed to be specifically adapted for high light level, photopic, vision. The retinula cells and associated rhabdoms extend the whole length of the retina from the basement membrane distally to the apex of the cone. The ommatidia are surrounded along their whole length by a continuous pigment sheath, within which little or no pigment migration occurs under the influence of light.

ii) Superposition eyes, in contrast, are found in nocturnal insects, especially Coleoptera and Lepidoptera, and are believed to be specially adapted for low light level, scotopic, vision. In such eyes retinula cells are short, extending only about one third of the distance from basement membrane to the cones. Marked pigment migrations occur in these eyes when the animal is moved from light into darkness and vice-versa.

The basic structural unit of the compound eye is the ommatidium, which is typically divided into two anatomically and physiologically distinct regions:-

1. A distal dioptric system of corneal lens and crystalline cone.

2. A more proximally situated photoreceptor region, the retinula, consisting of a varying number of elongated sense cells arranged radially around an axial structure, the rhabdom. Each sensory cell is a primary sensory neuron terminating proximally in an axon which passes through the basal limiting membrane of the retina to make complex synaptic connections within the optic lobe.

1. The Dioptric Mechanism

a) The corneal lens is a modified region of the general body cuticle. It is transparent and colourless, and in species of Lepidoptera and Coleoptera is extended proximally and functionally replaces the crystalline cone.

In many species the frontal surface of the cornea is covered with minute nipples arranged hexagonally with approximately 0.2μ between the centres of adjacent nipples (Bernhard and Miller 1962). Two types of experimental investigation, first using microwaves with scaled models of the cornea, and second by studying the reflecting properties of corneas with and without nipples (Bernhard, Miller and Møller 1963-1965, Miller, Bernhard and Møller 1964) have shown that the nipple array decreases reflection of incident light, and thus increases its transmission over a broad wavelength range. The reduction in reflectance has a camouflage effect.

b) The crystalline cone lies just proximal to the corneal lens. It is produced by the intracellular secretion of four large cells, the Semper cells. Four types of crystalline cone are recognised (Grenacher 1879; Kirchoffer 1910) and are classified into the eucone, pseudocone, acone and exocone types (see Imms 1960) on the type of the Semper cell secretion.

In superposition eyes the Semper cells always produce a tapering transparent filament, the crystalline tract or axial filament, which traverses the distance between the base of the "cone" and the underlying

rhabdoms.

2. The Retinula

Most ommatidia have eight retinula cells arranged like the petals of a flower around the ommatidial axis. In many insects one or two of these cells are smaller than, or eccentrically placed relative to the other retinula cells. In the locust the retinula contains one or two eccentric, or basal retinula cells with six or seven normal retinula cells, making a complement of eight cells per ommatidium (Horridge and Barnard 1965). In some insects the eccentric cells contribute a short rhabdomere to the formation of the rhabdom in the locust, however, the basal retinula cells do not have rhabdomeres.

The retinula cells produce a strand of high refractive index, the rhabdomere, which is composed of a closely packed array of microtubules approximately 500^{o}A in diameter, extending radially from the surface of the cells toward the centre of the ommatidium (Fernández - Morán 1958; Goldsmith and Philpott 1957; Goldsmith 1962). At one end the tubules are continuous with the general retinula cell cytoplasm. The other end is closed and may come into close contact with adjoining rhabdomeres.

In an ommatidium the individual rhabdomeres form the rhabdom. In the insect eye two distinct types are found:-

a) In the open rhabdom of Diptera and aquatic Hemiptera the individual rhabdomeres in the ommatidium do not come into contact with one another. In these eyes a rhabdom-bearing eccentric cell often

lies on the axis of the ommatidium and is surrounded by the other cells. (Sato 1950).

b) In the more usual closed rhabdom eye the individual rhabdomeres are closely packed together to form a composite structure, often triangular in cross-section, with the microtubules arranged in the three major axes of the triangle. The possible significance of this arrangement in the detection of plane polarised light cannot be overlooked.

The boundaries of the individual rhabdomeres in the closed rhabdom are usually distinct, but in some cases actual fusion of the rhabdomeres may occur. In the retinula of the honey-bee, for example, fusion of the individual rhabdomeres in pairs results in a four-parted rhabdom surrounded by eight retinula cells (Goldsmith 1962).

The rhabdom is presumed to be the photosensitive part of the ommatidium, although there is no direct evidence underlying this presumption. Several features do, however, support this hypothesis:-

1. Its situation on the axis of the ommatidium.

2. Its known light conducting properties (Page 17).

3. Its microstructure is similar to that of other known photoreceptors e.g. the outer segments of vertebrate rods, (Wald, Brown and Gibbons 1962).

4. The analogous structure in the squid eye (Hagins, Zonana and Adams 1962) has been shown to be the source of the retinal illumination potential.

5. Fisher and Ron (1959) located Vitamin A, the precursor of rhodopsin, in the proximal ends of the ommatidia, i.e. in the region of the sense cells, in the superposition eye of Lepidoptera. Their additional histochemical evidence suggests that much of this Vitamin A is contained in the rhabdoms.

6. Further, evidence from the developing pupa of Bombyx (Eguchi, Naka and Kuwabara 1962) shows that the development of electrical responsiveness coincides with the differentiation of the rhabdomeres.

Pigment in the compound eye is contained in both the retinula cells and in distinct pigment cells which differ from the retinula cells in that they are not innervated and do not form rhabdomeres. The pigment in these sites is quite different chemically from visual, rhodopsin type, pigments, and there is no evidence to suggest it plays a direct part in visual excitation. White-eyed mutant flies, which have none of these accessory pigments, have a greater sensitivity to light than do normal red-eyed flies a clear indication that these pigments are shielding and absorbing pigments rather than pigments directly concerned with photoreception (Autrum, 1955).

Exner (1891) first showed that pigment in the compound eye altered its position in response to changes in light intensity. Pigment movements are generally much more marked in superposition than in apposition eyes (Exner 1891; Collins 1934; de Bruin and Crisp 1957; Bernhard and Ottoson 1960 a.b.) and sensitivity in superposition eyes has been shown to increase up to 1,000 times with dark adaptation

(Bernhard and Ottoson 1964. See also Bernhard and Ottoson 1959; Hoglund 1963; Bernhard, Hoglund and Ottoson 1963).

Miller (1958) showed that radial movements of the pigment granules in the retinula cells of Limulus during dark adaptation expose a clear region around the rhabdom. Similar movements are known to occur in several insects; e.g. Forficula (Jorschke 1914), Barbitistes (Uchida 1934) and Mantis (Friza 1928). In Locusta (Barnard 1964; Horridge and Barnard 1965) the clear region is formed by the radial migration of mitochondria which in the light adapted eye closely invest the rhabdom. This clear area, the palisade, or the "Schaltzone" of the old light microscopists, is claimed by Miller (1958) to "allow entering light easy access to the rhabdom." Horridge and Barnard (1965), and this thesis, contend that the palisade increases ommatidial sensitivity to off-axis rays by enhancing the properties of the rhabdom as a wave-guide.

In some arthropods photomechanical responses are more complex. In Artemia the rhabdom elongates during dark adaptation (Debaisieux 1944). Similar changes are reported to occur in the eyes of Notonecta (Ludtke 1953) and Culex (Sato 1950; Sato Kato and Toriumi 1957). These types of movement are presumed to widen the acceptance angle of rhabdoms to light at low intensity, and thereby increase their light gathering power under these conditions. For a more complete list of the photomechanical responses of the compound eye see Tuurala (1954).

2. Optics of the Compound Eye

a) The Mosaic Theory

The original theory concerning the optics of the compound eye, the mosaic theory, (Johannes Müller 1826; Sigmund Exner 1891), presumes that each ommatidium in the eye is sensitive only to light entering on, or at a small angle to, its optical axis, more oblique rays being absorbed by the sheath of screening pigments surrounding the ommatidium. Each ommatidium responds as a unit to the average intensity in its field and contributes a small area to the total image perceived.

Following his study of the pigment movements occurring in compound eyes under the influence of light, Exner (1891) proposed two basic eye types (Section A (1)) and discussed the image forming properties of both. He considered the dioptric system of both eye types to be a lens cylinder, i.e. its refractive index decreased in concentric layers from the axis toward the periphery. Such a system would effectively funnel near-axis rays into the receptor layers and reject more oblique ones irrespective of the refractive index of the surrounding medium.

In apposition eyes, Exner considered the length of the dioptric lens cylinder to be equal to its focal length. Such a lens cylinder would then form a small inverted and reversed image at its apex where it comes into contact with the retinula, and the apposition of all the images formed by the different ommatidia would produce the total image perceived by the eye.

In superposition eyes the dioptric system was considered to be

a lens cylinder twice as long as its focal length; light rays would then leave the base of the cone at the same angle, and in the same plane relative to the axis, as they entered. The image formed would then be erect, and because of the optical properties of the lens system as many as 30 neighbouring ommatidia could concentrate light onto a single site in the retina, when screening pigments were in the dark adapted position. Since each of the elemental images is formed by the superposition of the individual ommatidial images, the total image perceived is termed a superposition image. During light adaptation changes in the position of the pigments would re-establish ommatidial isolation, and the retinula would then receive rays from its own facet only. In this condition the eye would function in the apposition manner.

If the mosaic theory is correct visual acuity must be dependent upon the number of units surveying a defined area of the visual field, i.e. upon inter-ommatidial angle, and upon the aperture of the lens system, which theoretically limits its resolving power according to the telescope formula (Mallock 1922; de Vries 1956).

$$\Phi^{o} = 1.22 \frac{\lambda}{d} \text{ radians}$$

Where Φ = ommatidial resolving power

λ = wave-length of light

d = ommatidial aperture (which is proportional to ommatidial size).

Barlow (1952) argued that under the mosaic theory optimum acuity

must depend upon the best compromise between inter-ommatidial angle and ommatidial size, the only variable in the telescope formula for light of constant λ, and considered the limits to which visual acuity may be improved by increasing the number of ommatidia per unit area. In many insects he showed that the ratio of inter-ommatidial angle to ommatidial resolving power approaches unity. If, however, ommatidial size was further reduced in an attempt to increase the number of ommatidia per unit area, and hence visual acuity, diffraction at the apertures of such ommatidia would cause overlap of resulting images and thereby loss of acuity.

The mosaic theory assumes that each ommatidium looks out on its own bit of visual space which is bounded by, but not shared by, that of its neighbours. Theoretically ommatidial sensitivity to near-axis rays is restricted by the structure of the doptric system, its position relative to the photosensitive regions of the eye and by the action of the pigment sheath. Together these factors should establish both the visual field of the ommatidium and the amount of light it admits.

With such a system the idealised spherical eye would form a reduced image, with an undistorted point for point representation of the visual field. The greater the divergence from this regular type of eye, however, the less perfectly would the image match the object viewed. Differences in corneal curvature in various planes of the eye will introduce astigmatism, with ommatidial size influencing light gathering power and ultimate resolution of the image (Barlow 1952),

while axial divergence will determine coarseness in the image and the total extent of the visual field.

In contrast to the postulated one-to-one relationship between ommatidial visual field and inter-ommatidial angle of the mosaic theory, recent optical and electrophysiological measurements of the visual field of single ommatidia show that the angular extent of their visual field exceeds the inter-ommatidial angle. Overlap of the visual fields of adjacent ommatidia is therefore demonstrated directly.

The results of all the studies where the visual field of the ommatidium is defined as Δ p are shown along with values of inter-ommatidial angle (Θ°) for a number of animals in table 1. The final column of this table indicates the relationship between Θ° and Δp°.

Vowles (1964) showed that the dark adapted value of Δ p, measured by direct microprobing in retinula cells of Musca, is reduced by 30% during light adaptation. Histological preparations show a close correlation between this reduction and the redistribution of pigment in the ommatidium, which occurs in the first ten minutes of light adaptation. Studies of the corresponding change in acuity of Musca (McCann and MacGinitie 1964) (i.e. the increase in acuity during the transition from dark- to light adaptation), also show a ten minute time course compared to a three minute period necessary for the recovery of receptor sensitivity during dark-adaptation. These changes are indicated in Table 1.

Using optical methods, de Vries and Kuiper (1958) and Kuiper (1962)

Table 1

Animal	Inter-ommatidial Angle ($\Delta\rho^\circ$)	Visual Field ($\Delta\rho^\circ$)	$\frac{\Delta\rho^\circ}{\Delta\varphi^\circ}$
Drosophila	4.6° (3)	3.5° (3)	0.76
Calliphora	2.5° (2)		
	H 3.2°) (9)	3.9°	1.2
	V 2.7°)	3.1°	1.1
Musca	H 3.9° (8)	d.a. $6^\circ - 7^\circ$ (8)	1.5 - 1.8
		5° (7)	1.3
		l.a. $4.2^\circ - 4.9^\circ$ (8)	1.1 - 1.3
		3.5° (7)	0.9
	V 2.6° (8)	d.a. $3^\circ - 6^\circ$ (8)	1.2 - 2.3
		l.a. $2.1^\circ - 4.2^\circ$	0.8 - 1.6
		$7.7^\circ \pm 2.1^\circ$ (4)	1.4 - 2.5
Apis	H 2.8° (1)		
	V 1.4° (1)		
	2° (6)	6.8°	3.4
Limulus	$4^\circ - 15^\circ$ (10)	13.2 - 15.4	1.4 - 1.6
	6.6° (5)	11.1 (5)	1.7

Reference List

1. del Portillo 1936.
2. de Vries 1956.
3. Götz 1965.
4. Kirschfeld 1965.
5. Kirschfeld and Reichardt. 1964
6. Kuiper 1962.
7. McCann and MacGinitie 1964.
8. Vowles 1964.
9. Washizu Burkhardt and Streck 1964.
10. Watermann 1954.

(see also Autrum and Weidemann 1962; Weidemann 1965) observed that when a point source of light is moved in front of the eye, the individual rhabdomeres, in the open type of rhabdom characteristic of the Diptera (Page 6), are not illuminated equally but become bright one after the other as the light source traverses the ommatidial field. They were thus able to measure not only the visual field of the ommatidium but also of the individual rhabdomeres. Their results show that the single cells look at 3 - 4° sections of a total ommatidial field of approximately 8° with the fields of the single cells arranged so that the centre of one field is at the edge ofits neighbour. Thus when the eye is illuminated by a point source of light, a number of sense cells in different ommatidia are illuminated rather than all the cells in a single ommatidium. The number stimulated depends in this case upon the visual fields of single sense cells and upon the angle between them.

From such observations it appears that in the open rhabdom eye the individual sense cells may be the basic optical units of the eye, rather than complete ommatidia which the mosaic theory presumes. This view has a good deal of evidence to support it:-

1. The visual field of the rhabdomeres, in the open rhabdom, is smaller than the field of the ommatidium as a whole.

2. Each retinula cell has a discrete nerve which passes into the optic ganglion.

3. Histological studies of the lamina (Braitenberg 1966) in

Diptera show that the optic cartridge receives a single retinula cell axon from each of a number of neighbouring ommatidia. These axons represent cells illuminated by a point source from a different position in different ommatidia.

4. Specialised retinula cells are demonstrated (Burkhardt 1962; Autrum and von Zwehl 1964) for the perception of the wave-length of incident light.

5. It is possible that individual retinula cells are concerned with the detection of the plane of polarisation of incident light.

None of this evidence proves conclusively, however, that individual sense cells are fundamental in either the analysis of form vision or the perception of movement, as distinct from other parameters of the visual input e.g. colour or plane of polarisation. Behavioural indices of the insect's ability to evaluate movement (page 22) are in general agreement with the mosaic theory.

In closed rhabdom eyes individual cells must be capable of independent response if diffraction images are to be of any physiological significance (page 27); however, in a closed rhabdom which is functioning as a wave-guide, it is quite impossible to visualise independent rhabdomere visual fields. In contrast with the open rhabdom eyes, the individual rhabdomeres are so closely apposed to each other and may even be fused together that any field differences would be so slight that they could hardly be significant. Much of the energy transported by a wave-guide travels outside the conducting rod and as

the distance between individual rhabdomeres is smaller than the wave-length of light, much of the energy trapped within one rhabdomere must enter the others. With the exception of the notions of the diffraction theory, these factors are suggestive of the corporate function of ommatidia in resolving both form and motion, in the closed rhabdom eye.

b) Wave-guide theory

It is now generally accepted that the dioptric and rhabdom components of the ommatidium, because they have a higher refractive index (n = 1.5) than the surrounding medium (n = 1.33) must act as wave guides (de Vries 1956). On this theory, originally considered by Exner, but elaborated by de Vries (1956) and Kuiper (1962), light which enters the rhabdom is kept within it by internal reflection and energy can then be transmitted down the rhabdom though it is long relative to its diameter. This process can be directly seen to occur in sections of retinal tissue when observed from behind, and is the principle of the technique employed in calculating ommatidial visual fields by optical methods (p. 13).

It is clear that light rays at the rhabdom/cytoplasm boundary, i.e. at a boundary between a medium of high refractive index and one of a smaller refractive index, will be totally or partially reflected depending upon their angle of incidence to the normal, and upon the refractive differences between the media, following the laws of reflection and refraction at such boundaries: (See fig. 1).

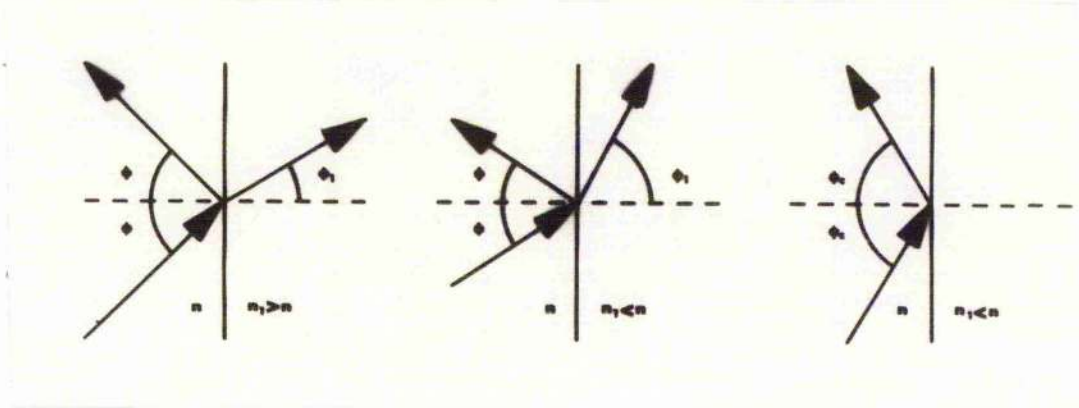


Fig. 1. Reflection and refraction of a light ray at an interface between two media of different index n and n_1

1. External reflection.

2. Internal reflection at an angle less than the critical angle.

3. Total internal reflection at the critical angle.

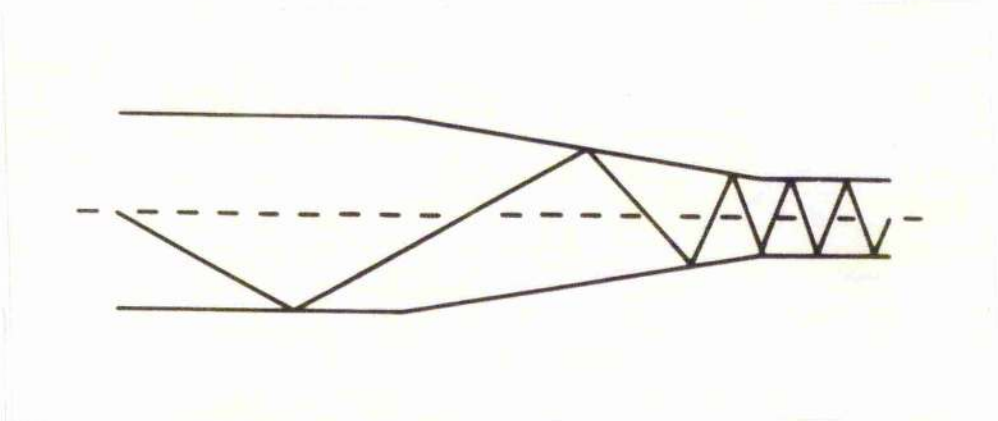


Fig. 2. The effect of a tapering wave-guide on the divergence of a light ray from its axis. (See text p. 21).

$$\frac{\text{Sin } \phi}{\text{Sin } \phi_1} = \frac{n_1}{n}$$

where ϕ = angle of incident beam to the normal

ϕ_1 = angle of refracted beam to the normal

n = refractive index of medium through which the incident beam travels.

n_1 = refractive index of the medium through which the refracted beam travels.

For the particular value of ϕ where $\phi_1 = 90^\circ$ ($\text{Sin } 90^\circ = 1$) there will be no refracted beam and light will undergo total internal reflection. This value of ϕ is called the critical angle (ϕc). At values of ϕ greater than this there will be no energy loss due to refraction and light captured in the dense medium will be transmitted through it.

As a consequence of the refractive relationship between the dioptric structures and the surrounding medium, light entering the ommatidium will be trapped within it by internal reflection to some extent depending upon its angle of incidence, and conducted into and down the photosensitive rhabdom, or in superposition eyes into the rhabdom via the crystalline tract which also has these refractive properties (de Bruin and Crisp 1957).

Depending upon the refractive index relationship, the proportion of incident light internally reflected will decrease with increasing divergence from the axis, and as a result the wave-guide will impart a directional sensitivity to the ommatidium.

The directional sensitivity of vertebrate retinal receptors, i.e. the Stiles-Crawford effect (Stiles and Crawford 1933), is known to have a similar basis. These authors demonstrated a directional sensitivity in the human eye which has subsequently been shown to originate in the retinal receptors themselves. The effect was demonstrated electrophysiologically by Donner and Rushton (1956) who showed a three- to four-fold change in the dark-adapted intensity threshold for a spike potential in the frog's retina over a 22^{o} range of directions. Tansley (1956) measured the brightness of transmitted light in the rod outer segments of the grass snake retina at various angles of transcorneal illumination. She demonstrated their directional sensitivity and concluded that total internal reflection was occurring with near-axis rays.

Several authors have suggested mechanisms to account for the Stiles-Crawford effect, and of interest is the work of O'Brien (1946, 1949, 1951). O'Brien built a model of a photoreceptor and studied its directional properties with radar waves (λ = 3.2 cms.) (Jean and O'Brien 1949). The results supported his theoretical considerations of the wave-guide properties of the receptor (O'Brien 1946).

Sidman (1957) and Barer (1957) measured the refractive indices of the various parts of retinal rods and cones and of the surrounding matrix. Barer gives indices of 1.405 - 1.410 for the rod distal segments and 1.387 - 1.396 for cones. Similarly he studied the refractive index of the mitochondria of the ellipsoid (R.I. = 1.390 - 1.398) and

of the cytoplasm of rods and cones (R.I. = 1.361 - 1.364). Using these refractive index measurements Enoch (1963) calculated from the Fresnel laws the proportion of incident radiation reflected at the point where an incident ray meets the boundary between the two refractive media. The importance of this study is that it shows the rapid fall-off of internally reflected light once the critical angle is exceeded.

O'Brien (1951) showed that a tapering wave-guide such as the cone ellipsoid has the effect of concentrating light into the smaller cross sectional area of the outer segment. This implies that a smaller amount of photochemical material will be required by the outer segment for a given response, in comparison to a non-tapered structure such as the vertebrate rod. This point is worth considering with reference to the arthropod crystalline cone which is also a tapering structure. However, as the decrease in cross-sectional area of a wave-guide is directly proportional to the increase in ray divergence from the axis, at some point this divergence will exceed the critical angle , total internal reflection will no longer occur, and the structure will no longer function as a wave-guide (Fig. 2).

Consideration of the rhabdom as a wave-guide has led to new theories concerning pigment migrations occurring in superposition eyes under the influence of light. Kuiper (1962) suggests that when the pigment comes into contact with the crystalline tract in light-adapting crustacean (superposition) eyes, the properties of the tract as a wave-guide are reduced and therefore a smaller proportion of incident

radiation will be propagated to the sense cells. A similar treatment is applied to the radial movements of mitochondria in the retinula cells of the Locust apposition eye (Horridge and Barnard 1965). Here the development, during dark adaptation of the palisade area of low R.I. around the rhabdom will change the value of the critical angle ϕ c (see above) so that ϕ c (light-adapted) is greater than ϕc (dark-adapted). This implies that a greater proportion of off-axis light will be propagated down a dark-adapted rhabdom than down a light-adapted rhabdom, and consequently ommatidial sensitivity to off axis rays will be increased with dark-adaptation.

If the optical system of the compound eye does function in this fashion, several features of the structure of the compound eye can be explained.

1. The shape and refractive structure of the crystalline cone and tract, and its relationship to the retinula.

2. The orientation of the rhabdom in the line of incident light rays and at right angles to the plane of images.

3. The position and migrations of pigments in superposition eyes, and radial pigment and mitochondrial migrations in apposition eyes.

4. The elongation of the rhabdom in several species during dark adaptation - which theoretically should increase ommatidial sensitivity to off-axis rays.

3. Optokinetic Responses and Motion Perception

In support of the mosaic theory, behavioural estimates of the visual

acuity of insects indicate a close correlation between the minimum stripe to which the insect will respond and minimum inter-ommatidial angle. Hecht and Wolf (1929) measured the visual acuity of the honey bee using as a criterion of acuity the smallest pattern repeat distance of a moving striped pattern to which the animal would respond optokinetically, and showed a close relationship between maximum acuity and minimum ommatidial separation. In areas of the eye where the interommatidial angle is larger, e.g. the periphery of the eye, they showed that visual acuity was correspondingly reduced. Similar experiments on Drosophila (Hecht and Wald 1934; von Gavel 1939) led to the same overall conclusion that visual acuity, in this sense, corresponded to the minimum inter-ommatidial angle.

It must be borne in mind, however, that optokinetic responses to striped patterns do not give a measure of visual acuity as usually defined. Optomotor responses involve both temporal and spatial parameters of the stimulus. Visual acuity as more usually defined depends upon spatial stimuli only.

Analysis of the optomotor response has, however, revealed significant information on the optical and physiological properties of the compound eye. Hassenstein (1951) devised an experimental set up where the turning tendency of the beetle Chlorophanus could be studied while its eye remained in a constant position relative to a rotating striped pattern. There followed a long series of papers (Hassenstein and Reichardt 1953-1956; Reichardt 1957, 1961, 1962; Hassenstein 1958;

Varju 1959) from which it seems clear that individual ommatidia are the smallest effective unit in determining and evaluating the motion of patterns. Reichardt (1957) postulated a mathematical autocorrelation model to account for the turning tendency, which shows the net turning response to be some function of the output of paired ommatidia. (See Reichardt 1962 for a fuller account). This model has subsequently been tested using a variety of techniques on a number of insects:- Drosophila (Götz 1964-1965); Musca (Fermi and Reichardt 1963; McCann and MacGinitie 1964); Schistocerca (Thorson 1964) without significant departures from the main theme.

These studies defined the visual input and took the overlap of ommatidial visual fields into account. In the evaluation of overlap the two important parameters are the inter-ommatidial angle (Δ°) and the visual fields of single ommatidia (Δ p) (see fig. 3) defined as the angle from axis where the light intensity must be doubled to give the same response as on the axis.

Götz (1964, 1965) assumed from data of Washizu, Burkhardt and Streck (1964) that the visual field of single ommatidia followed a Gaussian curve of width at half linear height of Δ p. Assuming also that the optomotor response makes use of all contrast in the visual field he considered the effective information available to each facet as the contrast between successive stripes, defining it as:-

$$\frac{J\text{max} - J\text{min}}{2 \text{ average } J}$$

Where J = the brightness of the stripes.

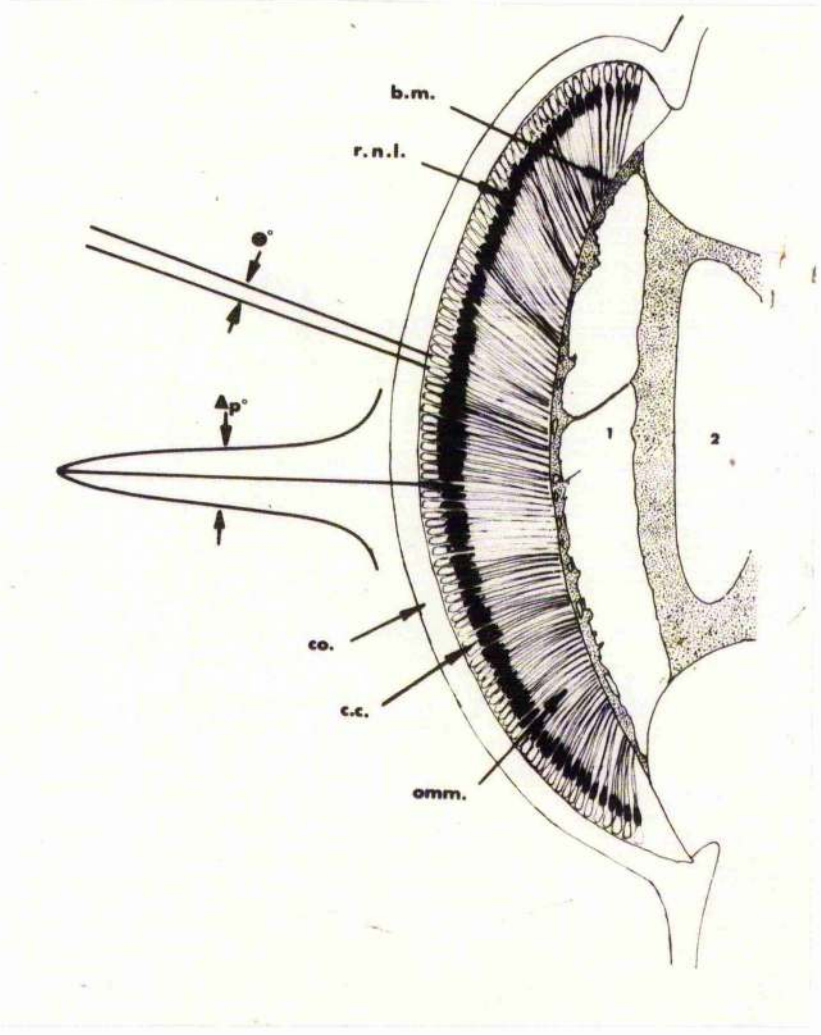


Fig. 3. Diagram of a section through the locust retina showing

Δp° - the visual field of a single ommatidium.

e° - the inter-ommatidial angle.

Other features shows are:-

b.m. - the basement membrane

c.c. - the crystalline cones

c.o. - the cornea

omm - the ommatidia

r.n.l.- the retinula cell nucleus layer

The post-retinal synaptic layers ((1. - the lamina
(2. - the medulla externa.

This function he called the input (me) of the optical system. Contrast in the pattern as seen by the ommatidium, i.e. the integral of all illuminated areas in the solid angle of visual field, he showed to be dependent upon Δp and upon λ, the pattern repeat distance, in the function;

$$\text{Contrast transfer} = e^{-(\frac{\Pi^2}{4\ln 2} \cdot (\frac{\Delta p}{\lambda})^2)}.$$

Thus function gives the fraction of the available contrast seen by the ommatidium and Götz was able to relate this to measurable optomotor responses from Drosophila which he called the output (ma) of the visual system.

When contrast transfer is high the optomotor response of the animal is maximal, i.e. me = ma and contrast transfer = 1. However if contrast in the pattern as seen by the ommatidium falls either by increase in Δp or reduction of λ the optomotor response will be some fraction of the maximum response.

Götz show that the relationship

$$\frac{ma}{me} = e^{-(\frac{\Pi^2}{4\ln 2} \cdot (\frac{\Delta p}{\lambda})^2)}.$$

Where: ma = contrast as seen by the ommatidium.

me = available pattern contrast.

When $\lambda > \Delta p$ contrast transfer is high, i.e. there is a maximum transfer of information from the pattern into the ommatidium. The reduction of contrast transfer is proportional to the reduction of λ until when $\lambda = \Delta p$, the contrast calculated from the above equation is equal to 0.027, that is contrast transfer falls to 2.7% when pattern repeat

distance equals acceptance angle at half height.

From this equation Götz can derive a value of Δp for an animal from its optomotor response. He postulates a minimum relationship $\Delta p/\rho = 0.62$ to 0.88 for maximum energy transfer per ommatidium, to enable successful optomotor response, and derives experimental figures of 0.76 for the behaviour of Drosophila. This figure is considered with other values of $\Delta p/\rho$ in table 1.

4. Diffraction Theory of Insect Vision

An apparent exception to the concept of a small Δp in insects is reported by Burtt and Catton (1961 a.b. 1962 a.b.) who showed that several species of insect have a better ability to resolve the motion of stripes than is indicated by behavioural experiments. In Locusta and in two species of Diptera, they recorded ventral cord spike responses to the motion of striped patterns, where the angular separation of stripes was as small as 0.3 degrees. This gives a value of resolving power for these eyes which exceeds the theoretical resolving power of a single ommatidium calculated from the telescope theory (page 10). Actual observation of two point sources through an eye slice of the locust gave values of the single facet resolving power of $1.08 \pm 0.03^{\circ}$. Where $d = 32\ \mu$ and $\lambda = 560\ m\mu$ (Burtt and Catton 1962b). The animal's measured sensitivity to stripes must therefore depend on a resolution which can only be obtained by combination of light from more than one facet. In support of this theory Rogers (1962) showed that a regular array of apertures will produce a series of images of a regularly

repeating pattern by a process of diffraction. Burtt and Catton show that the resolution of the diffraction images in the locust increases with depth in the eye from 1.08° for the first image to 0.35° for the fourth, (Table 1 page 65 Burtt and Catton 1962a), and conclude that the animal's high sensitivity to pattern motion must depend upon its ability to resolve these higher order images.

This theory is questioned on several grounds.

First, it is unlikely that sufficient light is able to penetrate the ommatidial pigment sheaths to form higher order images. Although Burkhardt (1962) postulates that some light of long wavelength (λ =616 mμ) can penetrate the pigment sheath at normal intensity in Calliphora, light of shorter wavelength is usually absorbed. Thus it is unlikely that diffraction images will be formed in apposition eyes unless very high contrast exists in the visual field.

Secondly, the lateral spacing of the retinula is too coarse to resolve patterns at the level of the third image (Burtt and Catton 1962), and therefore to resolve patterns at this level individual retinula cells must be capable of independent response. However the ability of single cells to function independently in the resolution of patterns, as distinct from other parameters of the stimulus, still remains to be shown for the closed rhabdom eye (page 16.).

Third, the formation of such images depends to some extent upon a homogeneous medium in the receptor layer (see however Rogers 1962). Inclusions in the cytoplasm of the retinula cells such as pigment grains,

mitochondria, endoplasmic reticulum, or regions of different refractive index within the eye as a whole are bound to cause dispersion of light and the disruption of any image.

Fourth, if the dioptric system does function as a wave-guide, there will be no effective aperture, as understood in the telescope theory which depends upon light spreading out behind the aperture and falling on a receptor device at some distance behind it.

Fifth, although the opposite point was argued (Burtt and Catton 1962a) it seems unlikely that compound eyes would evolve with longitudinal retinas if the perception of images of any type was of physiological importance. The diffraction theory must presume that the insect can 'focus' on specific levels of the retinula cells, and disregard intensity fluctuations caused by lower order images. It is more likely that receptor cells in the closed rhabdom eye are sensitive to changes in the net intensity in the rhabdom as a whole, as no differences have been noticed in the intra-cellular response of locust retinula cells at different levels (Scholes 1965).

Sixth, if light is able to traverse the retina to form these images, then shoulders should appear on the acceptance angle curves at wide angles of acceptance, and these are not commonly found. (page 63).

Palka (1965) has put forward an alternative explanation to account for the high resolution found by Burtt and Catton. He showed that when fine stripes are moved across a window of the type Burtt and Catton used the position of the window has a profound effect upon the recorded response. He was able to record giant fibre responses from the locust

ventral cord when stripes subtending 1° were passed across a window whose sides were parallel to the stripes. If, however, the edges of the window were not parallel to the stripes, the response falls and is indistinguishable from the spontaneous activity of the nerve cord when the edges of the window subtended 30° to the stripes. Palka, therefore, interprets the high resolution found by Burtt and Catton as arising from an effective change in the window position when the stripes are moved, i.e. from a lower spatial harmonic which the eye can resolve.

In 1965 Burtt and Catton repeated their experiments and again found a high resolution to rotating radially striped patterns. However the harmonics of the motion of such a pattern (i.e. a pattern with a continuous variation of λ from the centre to the periphery) will be quite considerable unless it is precisely made and accurately centred on the rotating disc, and could greatly influence the results. Apart from the observations of Burtt and Catton, high values of resolution can be explained more readily by the insect's high sensitivity to intensity changes. (p. 29).

5. Lateral Inhibition

The equations of contrast transfer (Götz 1964, 1965) seem adequate to account for behavioural and electrophysiological measurements of effective information transfer per ommatidium in the insect eye. In behavioural experiments, however, the test input is rarely confined to the single ommatidium. More usually optomotor behaviour is seen as a result of the summed inputs of every ommatidium in the eye. In this

situation lateral inhibitory interactions, if present in insects, will be an additional consideration in the equation of information transfer, which the Götz theory does not take into account.

A lateral inhibitory system has been amply demonstrated in the Limulus eye (Hartline with Ratliff 1956; 1957, 1958 and with Miller 1958). Under stationary conditions, spikes generated in the Limulus optic nerve are linearly related to the log of light intensity as long as only one ommatidium is illuminated, (Hartline and Ratliff 1958); however, if more than one ommatidium is illuminated the individual responses are mutually inhibitory and the total response of the optic nerve is not the arithmetic sum of the inputs. The more intense the stimulation of individual ommatidia the greater is their inhibitory effect upon adjacent units, and as a consequence differences in light distribution across the eye are exaggerated.

Reichardt (1961) and Reichardt and MacGinitie (1962) (see Reichardt 1962) proposed a mathematical model of the transfer properties of the whole eye, which implies that the lateral inhibitory system can reverse the effects of dioptric overlap and increase visual acuity. If reversal is complete, there will be an exact representation of the visual field in the optic nerve, and in these circumstances visual acuity will be limited only by receptor density.

The Limulus type of contrast-sharpening system, mediated through a sub-retinal nerve plexus, has not yet been demonstrated in insects, though lateral connections between ommatidia do exist which could be of possible inhibitory significance (Cajal and Sánchez 1915).

Burkhardt and Autrum (1960) note that the size of the generator potential from single cells in Calliphora is reduced by stimulation of nearby parts of the eye. This must modify the production of spikes in the retinula cell axons, and may indicate an as yet unexplained type of retinal lateral inhibition (See also page 94).

B. Electrical Responses and Electrophysiology of Compound Eyes

1) The E.R.G.

Extracellular recording in the retinal layers of arthropods reveals in general a cornea-negative potential, the electroretinogram, largely dominated by, and reflecting the depolarisation of the retinula cells. The form of the E.R.G. is very varied. In Limulus and many insects (Hartline 1928; Bernhard 1942; Autrum 1950 - see also Autrum 1958) it consists of a simple negative-going monophasic potential. In other insects, notably flies and bees, nervous activity in the optic ganglia contributes to the wave-form particularly at the "on" and "off" of stimulation (Autrum and Gallwitz 1951). The E.R.G. is then a multiphasic potential and usually has a positive-going on-transient and at the end of stimulation an off-transient of opposite polarity.

Autrum suggested a classification of eyes into two groups, based upon the form of the E.R.G. which he associates with other physiological features:- Fast eyes - with multiphasic E.R.G.'s, high flicker fusion frequencies and rapid dark adaptation in comparison with slow eyes which have monophasic E.R.G.'s., low flicker fusion frequency and a slow rate of dark adaptation. The origins of the various components of the E.R.G. and the concept of fast and slow eyes is still the subject

of a great deal of controversy, and Autrum's analysis is not generally accepted (Ruck 1958, 1961 a,b,c.).

The S.R.G. has, however, been used in studies of the spectral sensitivity of insect eyes (e.g. Goldsmith 1960), and of sensitivity changes associated with light and dark adaptation (e.g. Bernhard and Ottoson 1960).

2. The Intra-Cellular Response

Intra-cellular responses have been recorded from retinula cells in the Limulus lateral eye and from a number of insect retinulae.

In the Limulus eye Hartline, Wagner and MacNichol (1952) succeeded in recording both spikes and slow (generator) potentials from the retinula cells. Their observations have subsequently been confirmed by Tomita (1956), MacNichol (1956), Fuortes (1958; 1959) and others. Impalement of these cells reveals a variety of electrical responses to a stimulating light. In one case the generator potential consists of both dynamic and static phases, is of large amplitude and at the peak of the dynamic phase may overshoot the resting membrane (Benolken 1961). Spike potentials recorded in such cells are generally of small amplitude or may be absent altogether. A second type of cell shows small generator potentials and small spikes, whilst a third type reveals small generator potentials and large spikes (MacNichol 1956).

Tomita (1956) suggested that the differences in the relative amplitude of slow and spike potentials could be explained if it was assumed that they originated in different parts of the cell. By recording intra-and extracellular activity simultaneously, he found

that the intracellular recordings showed positive-going slow potentials and positive-going spikes, while the extracellular response showed negative-going slow potentials and positive-going spikes. He explained these results by concluding that the slow potential originates from a conductance change in the membrane, and that the resultant outward flow of current at the base of the retinula cell initiates spike potentials in the retinula cell axon. Thus in intracellular recordings the relative size of slow and spike potentials will depend upon the position of the electrode relative to these sites.

In 1958 Fuortes proposed that responses showing large spike potentials originated in the eccentric cell only, and the generator potentials in the retinulae. Recently, (Behrens and Wulff 1965) have substantiated this by simultaneous intracellular recording from two cells in the same ommatidium, in conjunction with marking techniques. They show-

a) That responses from retinula cells tend to lack spike potentials.

b) Simultaneous recording from eccentric and retinula cells show that the generator potential arises in the retinula cell and after a short latency invades the eccentric cell, and may trigger its response.

c) Spike potentials tend to be synchronous, suggesting that there is only one spike-generating site in the ommatidium, presumably the eccentric cell.

The amplitude of the generator potential varies approximately as a function of light intensity. In very dim light the response

fractionates into a random series of miniature potentials or "bumps" (Yeandle 1958) which probably represent the receptor response to the arrival of a single photon at the receptor surface.

The intracellular responses of insect visual cells are to a large extent similar to those of Limulus retinula cells (Naka 1961; Burkhardt 1962; Washizu 1964). The response usually consists of a slow generator potential and spikes are rarely recorded except in the case of the drone bee (Naka and Eguchi 1962). The generator potentials are differentiated, as is in the case in Limulus, into dynmaic and static phases, both related in amplitude to the logarithm of light intensity. The dynamic phase has not been reported to exceed the resting potential.

By employing techniques similar to those of Tomita (1956), Naka and Eguchi (1962) show that when spikes are recorded the site of spike generation is anatomically distinct from that of the generator potential. It is possible that the usual inability to record spikes from insect cells may be due to the choice of a peripheral rather than a proximal impalement site (i.e. nearer to the retinula cell axons). The situation might, however be similar to that in the Limulus ommatidium.

Although the retinula cells are in close contact with each other, especially in the closed rhabdom, they are presumed to be electrically independent. However the work of Behrens and Wulff suggests a close electrical coupling between the retinula cells and the eccentric cell in the apposition ommatidium of Limulus. A similar conclusion must be drawn from the work of Kikuchi and Ueki (1965).

C. Specific points from the literature on the locust

1. Anatomy

Any account of the structural detail of the locust ommatidium must draw upon the electron-microscope studies carried out at the Gatty Marine Laboratory by Barnard and Horridge, (see Horridge and Barnard 1965, Horridge 1966).

The eye is composed of a regular array of eucone ommatidia of the fused rhabdom apposition type. The cones approximately 32 μ at their widest diameter (Burtt and Catton 1962a) and 60 to 80 μ long are divided, as in most insects, into four parts each clearly visible in histological preparations. Proximally the cone comes into contact with the rhabdomeres, and here each cone segment separates and is continued as a long rod shaped structure, 0.5 to 2 μ in diameter, to the basement membrane. These structures, called the cone roots have also been noted in Drosophila ommatidia and it has been suggested that they provide mechanical support for the ommatidium and hold each retinula directly beneath the corresponding cone, (Waddington and Perry 1960).

Pigment is found in two large primary iris cells, in pigment or glial cells surrounding the ommatidium, and in the retinula cells themselves. The pigment grains are most densely packed around the apex of the cone, i.e. where the cone runs between the distal ends of the retinula cells, and it is likely that any light refracted out of the cone at this point will be totally absorbed without entering neighbouring ommatidia. The position of pigment in these sites is unaffected by

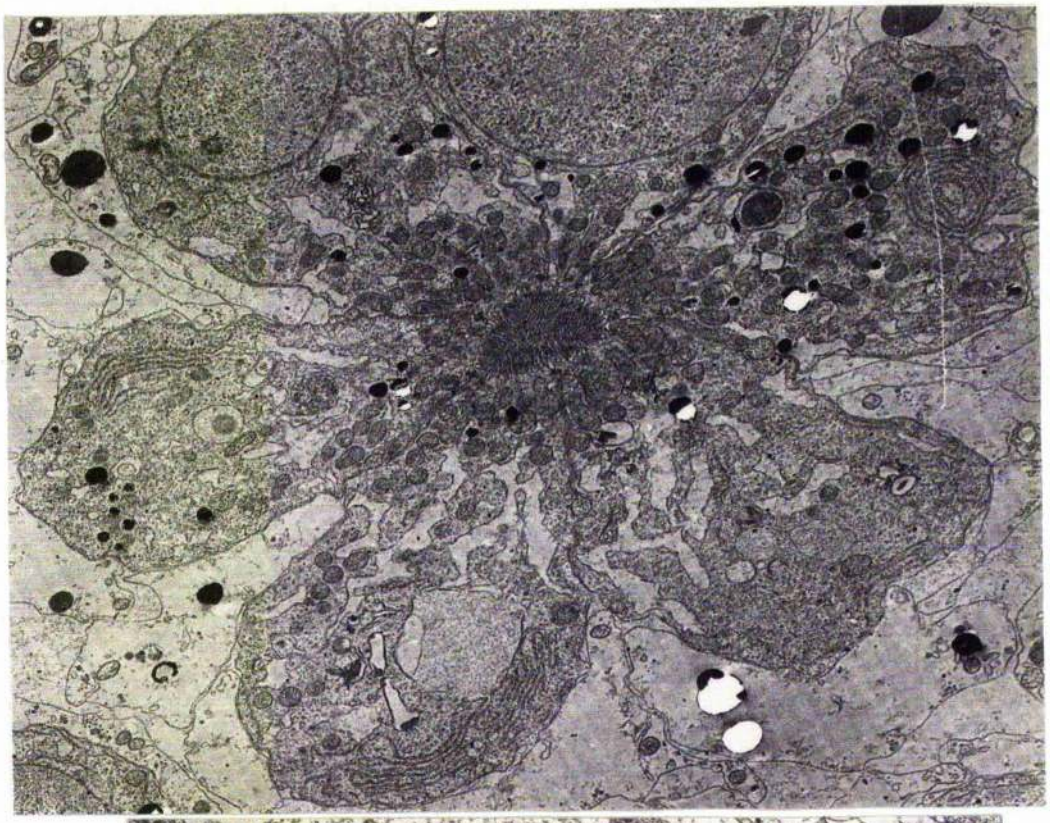
changes in light intensity.

The retinula cells are of the order of 300μ long, and 8 to 10μ in diameter. There is commonly but not invariably eight cells of which one or two may be rudimentary and lack rhabdomeres. Rudimentary or eccentric retinulae are smaller than the normal retinulae and are restricted to the proximal third of the retinula. The eccentric cells always appear in a position relative to the other retinula cells that is repeated in all ommatidia across the eye. Eccentric as well as normal retinula cells give rise to post retinal axons but the connections of these are not known.

In electron micrographs the retinula cell cytoplasm is seen to be rich in a variety of cytoplasmic inclusions, of which mitochondria are most evident. In the light-adapted eye the mitochondria closely pack around the rhabdom. During dark adaptation they migrate peripherally to expose a clear palisade area of fluid-filled vesicles around the rhabdom (Fig. 4). The rhabdomeres, although closely packed together in the rhabdom, are clearly distinct under the electron microscope, i.e. no fusion occurs between individual rhabdomeres. They are composed of microtubules approximately 0.1μ in diameter, whose membranes are continuous with the membrane surrounding the retinula cell. The microtubules of a retinula cell are orientated parallel to one another, and their lumina are continuous with the retinula cell cytoplasm.

Around the rhabdom, thickenings or desmosomes appear in the radial walls of the retinula cells. The significance of these is unknown.

a.



b.

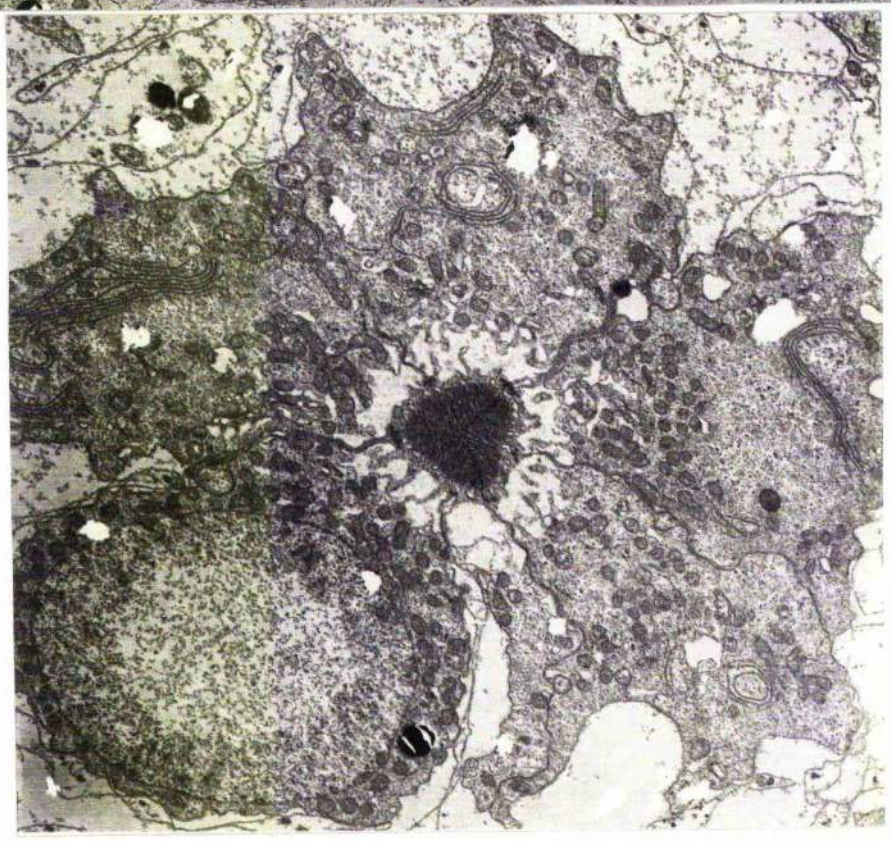


Fig.4. Electronmicrographs of locust ommatidia in (a) the light-adapted state, where the mitochondria lie close to the tri-angular rhabdom, and (b) the dark adapted state where the mitochondria have migrated peripherally to expose a clear palisade area around the rhabdom.

2. Electrical responses of the retina

Burtt and Catton (1956) describe multiphasic electroretinograms from the locust retina and discuss the possible origins of the various components. They observed that the polarity of the phases depended upon the position of the electrode tip relative to the cornea. Electrodes near the cornea record through an a.c. coupled amplifier negative going on-waves and positive going off-waves. As depth of electrode penetration is increased the amplitude of the defections is reduced, and at the basement membrane their polarity is reversed. Prolonged illumination shows a response plateau, at this depth, which is positive relative to the baseline in superficial recordings. At still greater electrode depths (i.e. in the optic lobe) the polarity of the waves is again reversed. Burtt and Catton (1962c) relate these observations to the potential profile of the locust eye, i.e. the standing potential of various regions of the eye relative to a corneal indifferent electrode.

The intracellular responses of locust retinula cells are discussed more fully in section 3A.

3. Movement Sensitivity

The ability to detect the movement of contrasting objects is well developed in the locust. Thorson (1964) showed that optomotor movements of the head will follow a sinusoidally oscillated coarsely striped pattern ($\lambda = 7^{\circ}$ or 45°) down to angles of 0.3° peak to peak oscillation. Similarly the visual memory of the locust is well developed. This is demonstrated (Horridge 1966) by allowing the animal to see the position

of a series of stationary stripes which completely fill its visual field. The light illuminating the stripes is then turned off. When the locust is receiving no visual input, the position of the stripes is moved through a small angle, and the light restored. The locust responds with a small optomotor response showing that it can perceive a movement of 0.1° which has occurred during a dark period of up to 10 seconds.

These experiments are not necessarily indicative of a high resolution, but demonstrate the locust's high sensitivity to the displacement of contrasting objects, the edges of which may not be clearly resolved.

4. Spike potentials in the optic lobes

Burtt and Catton (1960) recorded spike potentials extracellularly in the optic lobes of the locust. These authors classified the most commonly occurring unit types into "on-off" phasic units, with a dark discharge inhibited by light, "on" tonic units with practically no dark discharge and "pure on" units with no maintained discharge in either light or darkness. Recently this work was confirmed and extended, and now some 20 types of unit are recognised in the optic lobes (Horridge, Shaw, Scholes and Tunstall 1965).

5. Spectral Sensitivity

Very little electrophysiological or behavioural work has been done on the spectral sensitivity of locusts (see Uvarov 1966). Burtt and Catton (1962a) plot spectral sensitivity using the intensity

threshold for "off" and for "on" units in the ventral cord to various wavelengths of light incident at the eye. Their curve shows a maximum sensitivity at approximately 490 mμ.

More direct, intracellular, measurements of spectral sensitivity (Bennett and Dunstall unpublished) indicate three receptor types in the locust eye. The first, maximally sensitive at 520mμ, is a green receptor. The second, a blue receptor, is maximally sensitive at 431mμ. The third, a mixed receptor, is maximally sensitive at 431mμ with a second peak in the green (504mμ). However, whether locusts have true colour vision has not been demonstrated.

6. Other physiological properties

Estimates of ommatidial visual field and of inter-ommatidial angle in the locust eye have been made by Burtt and Catton (1954) and by Autrum and Weidemann (1962), and are shown below:

Interommatidial Angle Δφ	Total Visual field of Single Ommatidia.
H 2.4° (1)	21.3° ± 3° (1)
	4° ± 1° (2)
1° (1)	19.4° ± 2.6° (1)
	3.9° ± 1.0° (2)

1. Burtt and Catton 1954.

2. Autrum and Weidemann 1962.

Other properties of the locust compound eye are discussed with relation to the diffraction theory of insect vision.

2. MATERIALS AND METHODS

A. Animals

Adult individuals of Locusta migratoria were used throughout these experiments. The choice of this insect, rather than any other, was determined by several factors:

a. Individuals were available in large numbers from cultures maintained in the laboratory according to methods devised by the Anti-locust Research Centre, London. (Hunter-Jones 1961).

b. Locusts are large insects and are fairly resistant to experimental procedures and to dessication.

c. It had proved possible to record intracellularly from locust retinula cells.

d. Work on the optical and physiological properties of the locust eye had led to high values of resolving power and to controversial new theories of insect vision.

e. The anatomy of the eye had been closely studied, and changes in the refractive environment of the rhabdom during dark adaptation, which would allow a test of its wave-guide properties, had been postulated.

f. Satisfactory Ringer solution had been developed for the locust with the following composition, (Boyle 1953).

K.	10	m. - mole/litre
Na.	140	"
Ca.	2	"
Mg.	2	"
H_2PO_4.	6	"
$H\,CO_3$.	4	"
Cl.	148	"

B. The Eye Preparation

Though it is probably preferable to record from the retinula cells of a restrained but intact animal, movements of the retinal tissue associated with respiratory or jaw movements make this impractical in the locust. In the interests of stable recording conditions, therefore, all work was carried out on isolated heads. The resting and generator potentials recorded from retinula cells in isolated heads are similar to those recorded from the retinula cells of other insects mounted intact, e.g. Calliphora.

Eye slices were made with a sharp razor blade as indicated in figure 5. Part of the head was left attached to the eye, which made handling and accurate positioning of the slice in the stimulus beam easier. Also, leaving part of the head attached to the compound eye ensured that the sub-retinal tracheal plexus remained open for respiratory exchange; however, this has not been shown to be important for the viability of the slice. The brain was not directly disrupted by the plane of the cuts and anntenal touch reflexes often persisted for a few hours after setting up the preparation.

To ensure that the sensitivity of all preparations was constant at the beginning of an experiment, eye slices were taken from light-adapted animals, under normal room illumination, and placed in the experimental position for a dark adaptation period of 15 minutes.

The preparation remained in air throughout the experiment, and was kept moist through a bridge of filter paper soaked in Ringer's Solution.

To avoid possible artifact due to dessication the preparation was changed every two hours, although its electrical responsiveness was apparently unchanged over this time.

C. Apparatus

A perspex dish was constructed so that the eye slice rested on a strip of filter paper kept moist by a reservoir of Ringer's solution. The reservoir was earthed through an Ag-AgCl indifferent electrode so that the slice was virtually at earth potential. The strip of filter paper also served to keep the preparation moist (see above). No special arrangements were made to keep the preparation oxygenated.

Electrodes were made from Owen's Illinois type Vl - 161 glass tubing drawn to a fine taper on a home-made spring-activated puller. To protect the tips each electrode was placed inside a glass tube of slightly larger diameter and batches of a dozen were strapped by elastic bands around the circumference of a larger tube to form the fasicule (see Frank and Becker, 1964). Other elastic bands prevented the electrodes from falling out of the fasicule when it was moved.

The fasicule was boiled under vacuum in absolute methanol until all the air had been removed from the electrodes. After boiling, the electrodes were allowed to stand in methanol for at least 24 hours so that any remaining air bubbles went into solution with the methanol. The electrodes were then rapidly rinsed in distilled water and immediately placed in 3M KCl solution. They were left in this solution at room temperature for 24 hours for diffusive alcohol/electrolyte substitution

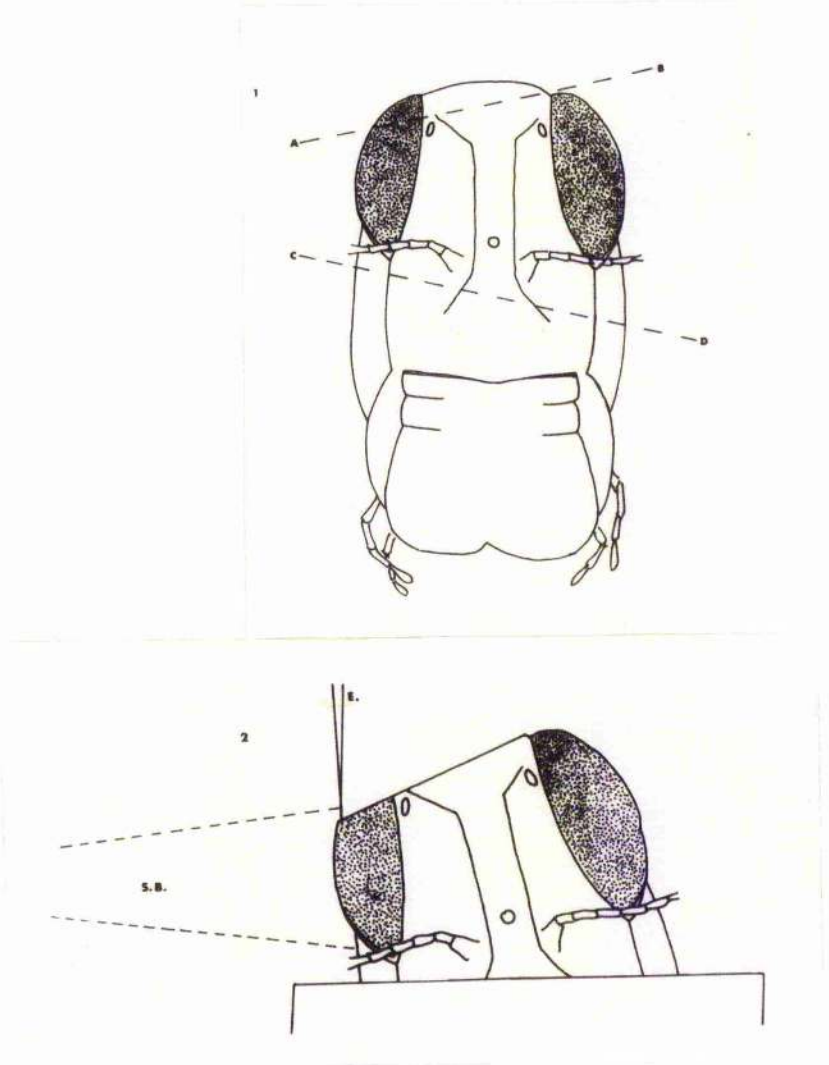


Fig. 5. The Eye Preparation.

The head was cut along the dotted lines A –B, C – D, (fig. 5.1), leaving part of the head attached to the eye (fig. 5.2)

E – electrode.

S.B – Stimulus beam.

to occur. This process could, however, be speeded up by incubating the fasicule at 20°C for 3 - 6 hours. Electrodes were not used after these times as KCl tends to etch and enlarge the tips. Electrodes were considered usable if they showed a resistance between 20 - 80 megohms measured in concentrated electrolyte with a 50 cycle bridge. Electrodes with a resistance either lower than or in excess of this range were not used.

When microelectrodes were to be used they were carefully washed in distilled water and dried to remove excess electrolyte. They were then mounted in a perspex holder which carried a co-axial lead to the pre-amplifier. The screening of this lead was driven (see fig. 6). A short length of silver wire made contact between the lumen of the microelectrode and the input lead. It was found unnecessary to coat the silver wire with AgCl. Junction potentials between the electrolyte or Ringer's solution and the silver electrodes could be matched to within a millivolt.

The headstage used was a unit gain cathode follower (Bak 1958, see fig. 6) built with slight circuit modification to suit the characteristics of a Mullard E80F used as the input valve. D.C. input resistance was set at 10 K Megohms and grid current could be adjusted to a lower limit of 10^{-12} amps. The input impedence could also be adjusted to high values by the capacity feed back loop on the input valve which effectively shortened the time constant of the amplifier so that it could follow high frequency transients. For the low frequency responses

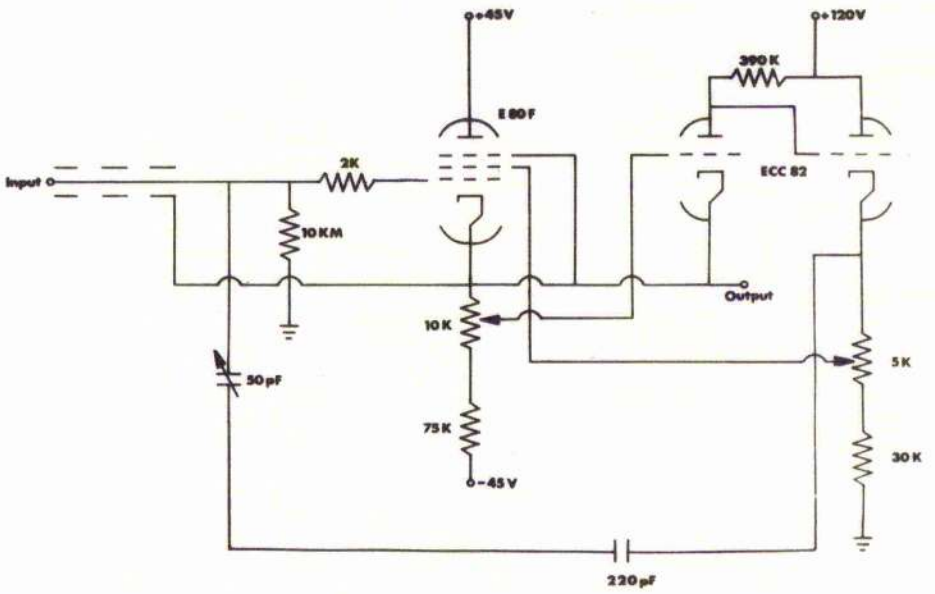


Fig. 6. Circuit diagram of the modified Bak unity gain cathode follower.

characteristically recorded from this preparation, however, it was unnecessary to compensate for the capacitative effects of the input lead and, in order to maintain a high signal-to-noise ratio, the capacity compensation facility was disregarded. Subsequent amplification and display were achieved by a Nagard oscilloscope (Type 311) with high gain directly coupled differential amplifiers (Type 311V). The signals were also displayed on a monitor oscilloscope (Nagard Type 1008) from which photographs were taken.

The diagram, fig. 7, illustrates the layout of the experimental apparatus.

b. Stimulating arrangements

The light source employed in the measurement of the visual field of single cells was a 36 watt tungsten filament bulb powered by 6 volts a.c. The bulb was enclosed in a tight fitting metal canister which was lined on the inside with black non-reflecting paper. An aperture of 3 mm. diameter drilled in the canister established a point source of light which subtended 0.5^{o} at the corneal surface 30 cms. away. No collimating or condensing lenses were used to modify the light path.

An electromagnetic shutter driven by a relay armature, occluded the aperture when the relay was in the relaxed position. The relay was activated by 50 volt pulses from a Tektronix pulse generator (Type 161) suitably modified to give pulses of sufficient current. The pulse generator was triggered to give a pulse after suitable delay by a negative sawtooth derived from the X amplifiers of the oscilloscope so

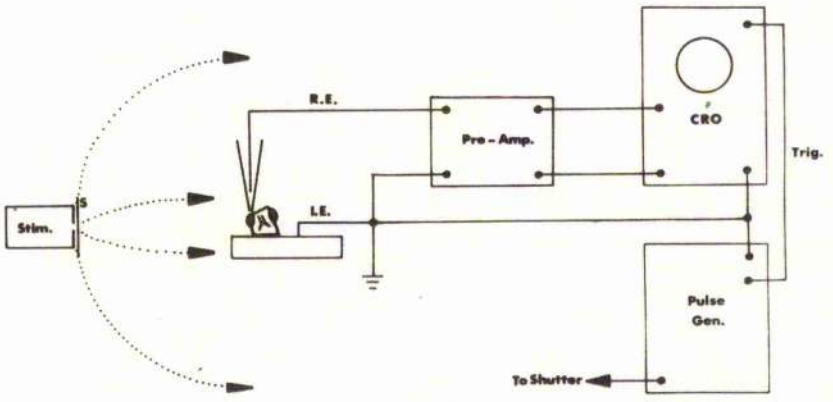


Fig. 7. Block diagram of the layout of the experimental apparatus.

C.R.O. Cathode-ray oscilloscope (Hagard, Type 311)

Pre-amp. Pre-amplifier (Bak).

Pulse Gen. Pulse Generator (Tektronix, Type 161).

I.E. Indifferent electrode.

R.E. Recording electrode.

S Shutter.

Stim. Stimulator.

The dotted arrows represent the movement of the stimulator.

that the shutter was synchronised with the time base of the oscilloscope. Light pulses of constant intensity, 10 msec. to 10 secs. in duration, could be delivered at frequencies varying from one per sec. to one per min, 100 msec, pulses at a frequency of one every 5 seconds gave constant responses from dark-adapted cells, i.e. at this frequency a light flash had no adaptive influence upon the cell's ability to respond maximally to a succeeding flash. This flash frequency was used in the estimation of the dark-adapted acceptance curve, to avoid anomalous results caused by uncontrolled adaptive changes.

In order to measure the light-adapted acceptance curve the frequency duration relationship of the light pulse was reversed so that after a 5 sec. period of light stimulation the light was transiently switched off. The dark interval could be varied until the cell response equilibrated to a minimum level. Alternatively the adaptive state of the cell could be altered by radiating the eye with light from a second source placed 5° above the axis of the cell under observation.

In both cases the amplitude of the response to the "on" of the light pulse was used as a measure of the effectiveness of the stimulus. The stimulating light was mounted on the bearings of an astrocompass so that the light could be moved independently in both horizontal and vertical planes of a sphere subtended from the eye. The direction of movement was calibrated to 0.5° about the poles of the sphere.

Acceptance angles were measured in 20° arcs, i.e. 10° to either side of the defined axis in both horizontal and vertical planes.

The angular position of the axis of the cell (see p63) was called zero degrees and the 10^{o} partial arcs, in both planes, were called positive and negative horizontal and vertical flanks depending upon the position of the light source relative to the axis and to either the front or top of the head. (see fig. 8).

5. Limitations of the technique

There are two limitations in the angle measuring technique employed in this study. First, in order to estimate the visual field of single cells accurately the stimulating light should move through an arc centred upon the facet of the ommatidium containing the retinula cell under electrical observation. It was, however, impossible to centre the light in this position because the exact facet among many could not be determined. It was found more practical to centre the light on the mid-point of the head, i.e. about 0.3 cms. behind the cornea. All angles measured with the light centred about this position will be slightly smaller than the actual angle of acceptance; however, the correction factor is so small (x 1.02) that it has been disregarded.

The second limitation of the angle-measuring technique employed involves the fact that the device used was calibrated about the poles of the sphere described by the motion of the lamp. With such a device angular readings on the scale will only be accurate for readings taken on one of the greater circles of the sphere, unless a correction factor is used:

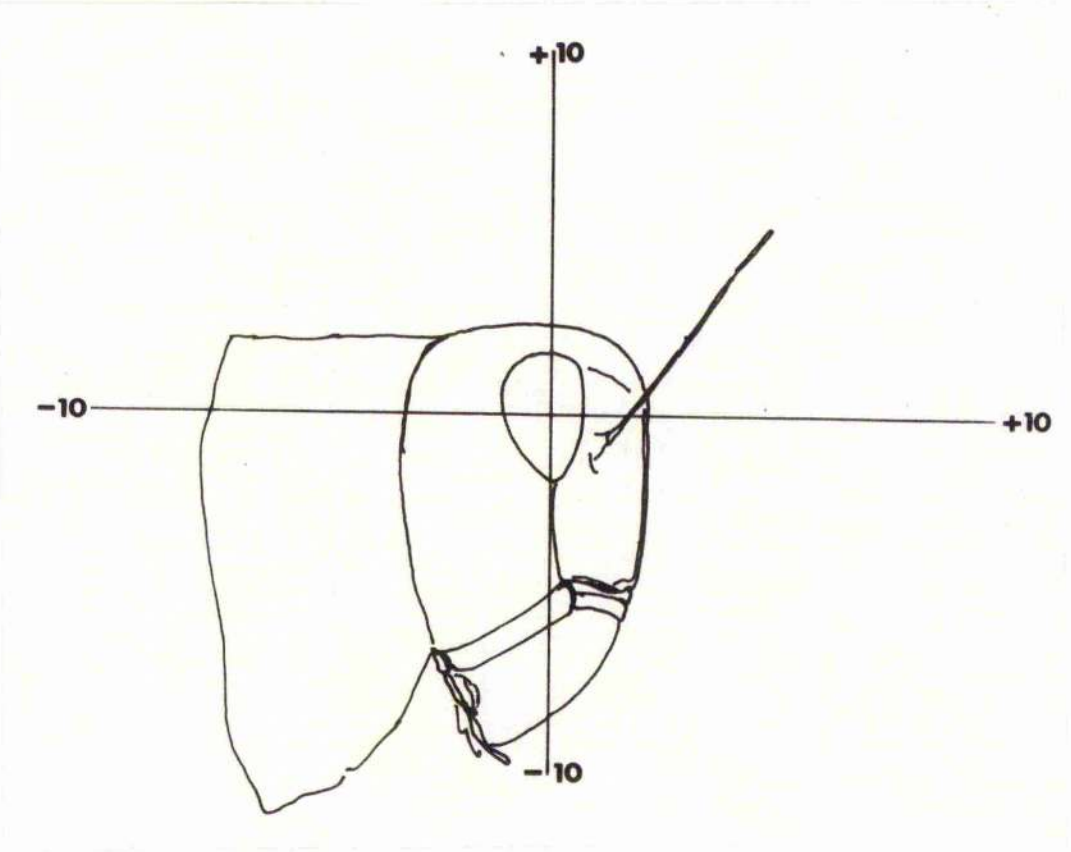


Fig. 8. Diagram defining the +ve and -ve flanks of the acceptance distribution, and the horizontal and vertical planes of the head.

Actual angle = measured angle x cosine of the angle of the light source from the greater circle (see Burkhardt 1965). This correction factor need only be applied to angles measured in a horizontal plane of the head which is not the equator. All vertical movements of the device used described arcs which were greater circles.

Although absolute co-ordinates of the light source on the axis of the cells were not measured, recordings were taken only from cells lying either under the equator or within 10 - 15° of this position. At the maximum position, i.e. 15° away from the equator, the correction factor = 0.9659, i.e. 10° measured about the poles, in a plane 15° from the equator, would equal 9.659°. Acceptance curves in the horizontal plane of retinula cells lying at the equator will appear to be narrower than equivalent curves from cells in other positions; however, the differences are so slight that they will not significantly alter the results presented in this thesis.

F. Histological Methods

Separate fixation and embedding techniques were used to study the gross and the fine anatomy of the retinulae.

For the measurement of inter-ommatidial angles small slices of the eye were fixed in Bouin's solution for 24 hours. After appropriate alcoholic dehydration they were cleared in cedarwood oil, washed in benzene, and embedded in high melting-point paraffin wax. 10μ sections were cut in both planes and mounted in Canada balsam. Inter-ommatidial angles were measured from photomicrographs (fig. 29) of these sections.

In order to measure the development of the palisade during dark adaptation, locusts were placed in dim red light and thin slices cut from their eyes after various periods. The slices were fixed for 2 hours in a 1:1 mixture of 2% OsO_4 and Ringer's solution. There was no attempt to buffer this mixture. The material was dehydrated in an acetone series and transferred to Araldite from 100% acetone. After 24 hours at room temperature the tissue was placed in fresh Araldite and cured at 50° C for three days. When the Araldite had cured small blocks containing the tissue were mounted with sealing wax on small stubs of dowel. This allowed accurate orientation of the tissue so that truly transverse sections could be cut. After trimming, the blocks were cut on a Porter-Blum Mkl. ultramicrotome. Sections were cut at 0.25 μ and stained with saturated aqueous toluidene blue diluted 1:1 with saturated aqueous borax. No control was made to estimate the extent of the dark-adaptive change in the ommatidium which might occur during fixation.

C. Stimulus Intensity Calibration

No absolute measurement of the intensity of the stimulating light was made. The unattenuated intensity of the source was termed 100% intensity.

A series of Wratten neutral density filters were calibrated in the experimental set up using an intensity-linear phototransistor (Texas Type Ls 222).

Table 2 lists the transmission of the filters and their calculated optical density.

Filter name.	Optical density.	Transmission.
0.1	0.12	76%
0.2	0.23	58%
0.3	0.36	42%
0.4	0.46	34%
0.5	0.58	26%
0.6	0.70	20%
0.7	0.78	16%
0.8	0.86	14%
0.9	0.95	11%
1.0	1.08	8%

3. RESULTS

A. General Observations

As a micro-pippette is moved transversely through the retinula cell layer, standing potentials of up to -60mV may be recorded over large areas. These potentials sometimes indicate the penetration of a visual cell; however, other times they may originate in non-visual cells or in extracellular areas. The form of the E.R.G. from these areas is very variable and usually takes the form of a slow, persistant wave form, graded with intensity up to 5mV amplitude at the "on" of high intensity stimulation.

In areas where the standing potential is approximately zero, the E.R.G. is usually a monophasic negative potential, that from its wave-form and sign is identified as the local extracellular derivative of the retinula cell response.

Penetration of visual cells by an electrode is not always obvious. Often penetration of what appears to be a retinula cell proceeds in a step-wise fashion, that is, further slight advance of the electrode leads to successively higher values of resting potential. Thus with care it is possible to improve an initially low value of resting potential up to values of -40 to -50mV without apparently damaging the cell.

Although no quantitative study of the resting potential of single cells was made, it is apparent that locust retinula cells are similar to other arthropod visual cells in this respect. Values for locust cells vary between -10 to -60mV as judged by the potential rise when

the cell died or when the electrode was mechanically removed from it. The largest value of resting potential recorded for a cell in the course of this work was -54mV, and the majority of observations are taken from cells with values of resting potential greater than -35mV. In these experiments the characteristic wave-form of the de-polarising response of the cell to light stimulation was the only criterion used to recognise a successful penetration since no marking technique was employed to directly verify the recording site. Fig. 9 illustrates the response of a single cell to high intensity stimulation. The initial transient dynamic phase and the sustained, smaller, static phase are similar to those recorded from Limulus and from other insect eyes. The relative prominence of these phases, however, varies from cell to cell, and no evidence has been put forward to account for this variation.

The maximum amplitude of the generator potential response to a light of constant intensity appears to vary in a simple fashion with the magnitude of the resting potential (Fig. 9 b-d) in agreement with Naka's (1961) observations, and it is unlikely that complete depolarisation of the retinula cell membrane occurs during the response to high intensity stimulation.

The slowly-decaying after-depolarisation which persists after the end of intense stimulation (Fig.9a) may represent a continued membrane permeability after the cessation of the stimulus. Alternatively the light-induced change in membrane conductance shown for Limulus and Calliphora cells (Fuortes 1959, Washizu 1964) may be sufficient to

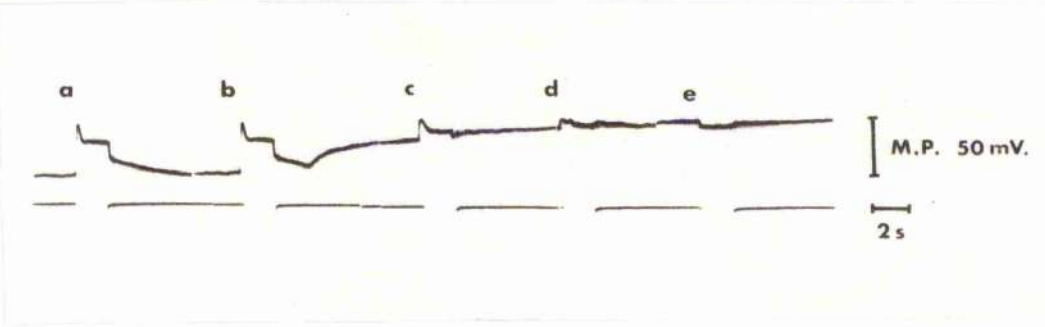


Fig. 9. Relationship between the amplitude of the generator potential and membrane potential.

a. The response to high intensity stimulation at full membrane potential.

b – d. The electrode was slightly withdrawn from the cell (end of response b). The response to equal intensity flashes is now smaller, c and d.

e. Extracellular response (E.R.G.).

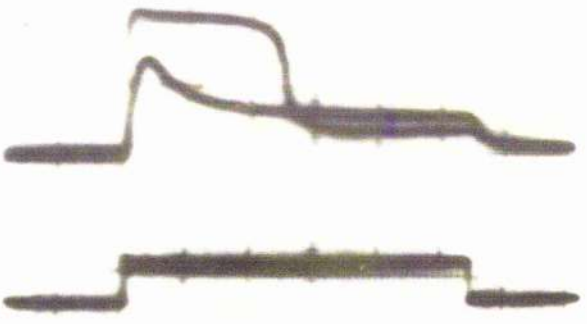


Fig. 10. The effect of continued dark adaptation upon the generator potential. (See text page 59).

change the ionic medium in the extracellular space immediately around the cell which will take some time to re-equilibrate to the normal resting level. The after-depolarisation is generally lost with reduction of light intensity.

The wave-form as well as the amplitude of the generator potential response to high-intensity stimulation is dependent upon the state of dark-adaptation of the eye. This is shown in Fig. 10 where, in a well dark-adapted cell the potential remains at the peak of the dynamic phase for as long as 1.5-2 seconds before it falls to a sustained level lower than the static phase of the same, more light-adapted, cell.

The amplitudes of both static and dynamic phases describe sigmoidal curves when plotted on a logarithmic scale of stimulus intensity (see sec. 38). The curve for the dynamic phase has a steeper slope than that for the static phase but both saturate at approximately the same stimulus intensity, and further increase in intensity has no effect upon the response amplitude.

At very low levels of stimulus intensity the response of a cell occasionally resolves itself into a series of randomly distributed waves or miniature potentials. The amplitude of these potentials is irregular and small with a maximum of approximately 1.0 to 1.5mV. Their frequency is linearly related to the light intensity up to the level where they fuse into a sustained potential, (Scholes 1965 a).

Unlike the Limulus eye preparation spikes are rarely recorded from locust or from other insect visual cells, with the exception of

the drone bee, where Naka and Eguchi (1962) recorded action potentials of up to 20mV. In a small number of the locust cells studied, however, it was possible to discern spike potentials just above the recording noise level. It is possible that spikes would have been recorded more frequently had penetration of the visual cells been made nearer to the basement membrane, i.e. nearer to the probable site of spike initiation, or had the capacity compensation facility of the Bak headstage been employed (see page 47).

B. Amplitude of the generator potential and light intensity

Fig. 11 shows the response of a single cell to light stimulation of decreasing intensity over 3 orders of magnitude. At low intensity (intensity 0.1 and 1.0 of the figure) the response of the cell is a small monophasic depolarising potential which, after a short latency, is maintained for the duration of the light flash. At higher intensities the potential resolves itself into two components, the dynamic and static phases, both graded with intensity. Differences in the relative amplitudes of these phases becomes more marked with increasing light intensity (10 and 100 in the figure). The amplitude of the generator potential saturates at intensities 10 to 100 times that of the maximum intensity shown in the figure.

Fig. 12 shows the relationship between the amplitude of the initial depolarisation of the cell; i.e., either the dynamic phase at high intensity or of the rising phase of the sustained potential at low intensity, and the relative intensity of the stimulus. The figure

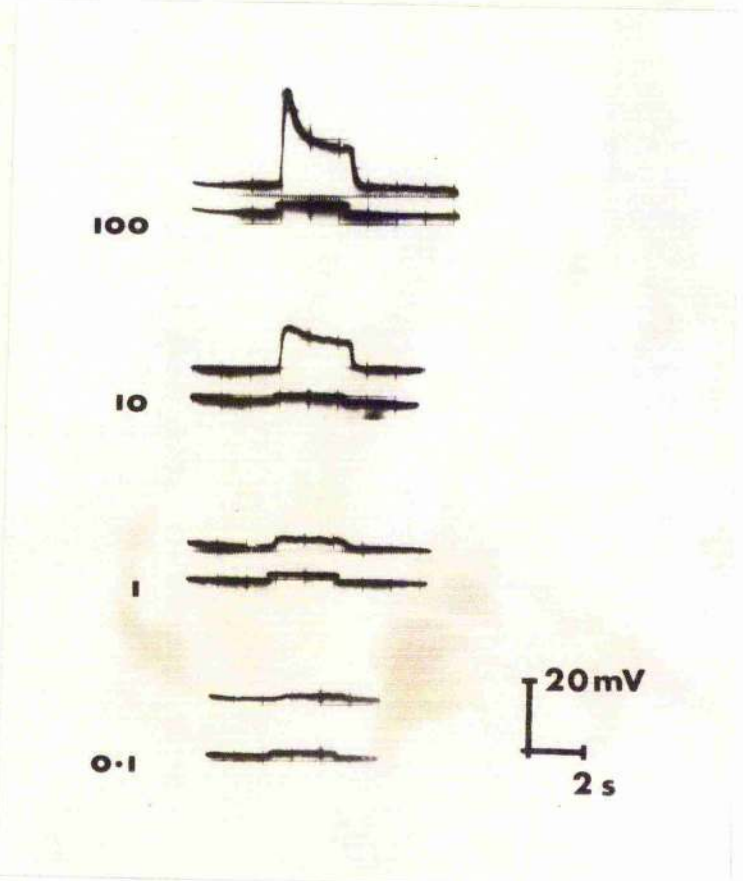


Fig. 11. Intracellular response of a single cell to a light source, of decreasing intensity, on its axis.

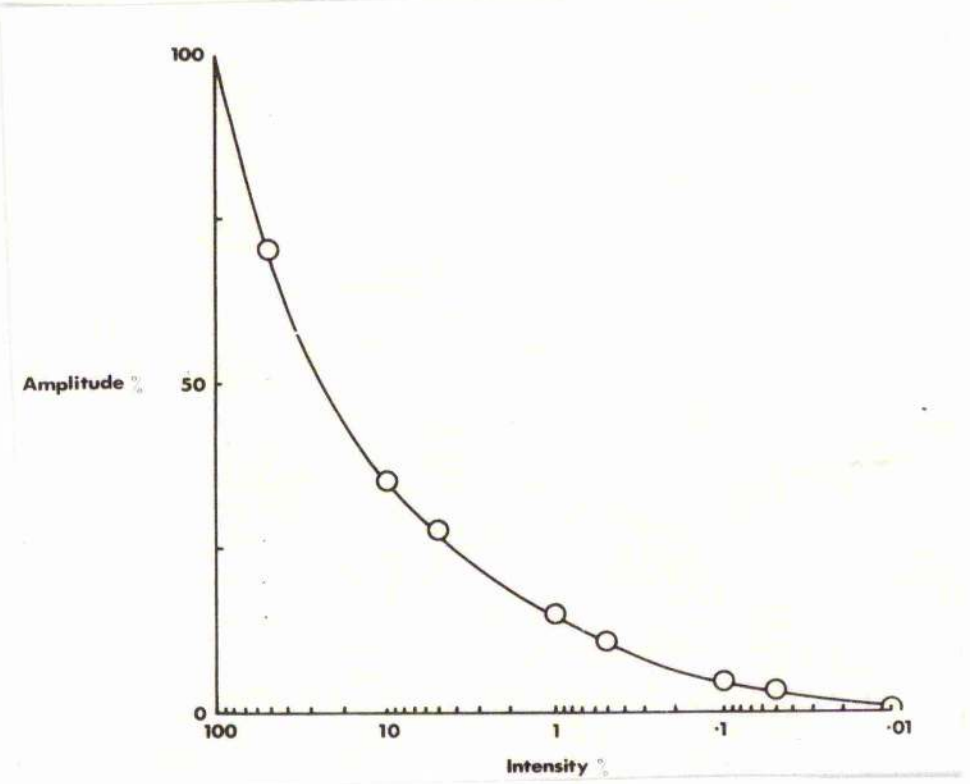


Fig. 12. Relation between the relative amplitude of the receptor response and the relative intensity of the stimulating light.

showing this relationship in 20 cells, both light - and dark-adapted, was constructed by calling the maximum response of the cell 100% response and scaling all other response amplitudes to this.

C. The Visual Axis

The amplitude of the intracellular potential depends upon the position of the light source relative to the particular cell under observation, i.e. upon the angle of stimulus incidence. By appropriate two dimensional movements of the light, the amplitude of the receptor response can be improved until the position eliciting the maximum potential is found. This position was defined as the visual axis of the cell if subsequent small movement of the light in any direction caused a reduction of the response amplitude. The progressive reductions of potential with increasing angle of stimulus incidence describe the amplitude inclination curves (see sections 3:D, 3:E.).

Light of low intensity was used during the search for the visual axis to avoid possible membrane saturation effects (see p. 59) which would lead to a flattening of the amplitude inclination distribution about the axis, and make it impossible to determine the position of the axis accurately.

In the majority of cells studied it was possible to use the visual axis, as defined, as a constant reference point to which all angular and potential measurements could be related. Approximately 10% of all cells studied, however, had either large, markedly assymetrical visual fields or more than one axis, i.e. there were two positions separated

by 2° - 5° on the amplitude/inclination distribution where the maximum potential could be recorded. Some cells showed secondary minor peaks in their amplitude/inclination distribution, separated from the major peak by 2° to 8°. In general, the nearer these second peals were to the major axis, the greater their amplitude. A cell with more than two peaks was observed on one occasion.

In a small number of cells the axis changed its position by 1 - 2° during an experiment, and either became stable in a new position, or continued to change its position, apparently following the traverse of the stimulating light. If, for example, the extent of the visual field of such a cell was studied in the +ve horizontal plane, subsequent re-study of the axis showed it had moved 1 to 2° in that direction. If the -ve flank of the visual field was then studied, the axis either re-established itself in its original position, or moved to the negative side of the original axis.

Measurements taken from cells which could not be related to a single stable axis were disregarded in the analysis of the amplitude inclination distribution (secs. 3:D and 3:E).

Relationship between visual axes

To establish whether single retinula cells in the ommatidium have either a common visual axis, or have different visual axes, the angular relationship between the visual axes of neighbouring retinula cells was measured. In the course of this work it was possible to make this estimation in 61 pairs of cells, where successful penetration of a cell immediately followed the death or loss of another.

The histogram of Fig. 13, shows the results of this study. The distribution of angles between the visual axes of neighbouring cells in the vertical plane is quite broad over the range 0.5° - 3.0° but has definite peaks between 1° - 1.5° and 2° - 2.5°. Although the head preparation was not always oriented with the horizontal rows of ommatidia at 90° with respect to the plane of the electrode advance, these peaks roughly correspond to the minimum inter-ommatidial angle in the vertical plane measured histologically (p104) or to a small multiple of it. In a small number of cells the relationship between the axes was smaller than the average inter-ommatidial angle in the central region of the eye. This may indicate successive penetration of two cells within the same ommatidium or penetrations of a cell on the dorsal side of an ommatidium from one on the ventral side of an ommatidium immediately above it. Two retinula cells with a common visual axis were never observed, although carefully looked for.

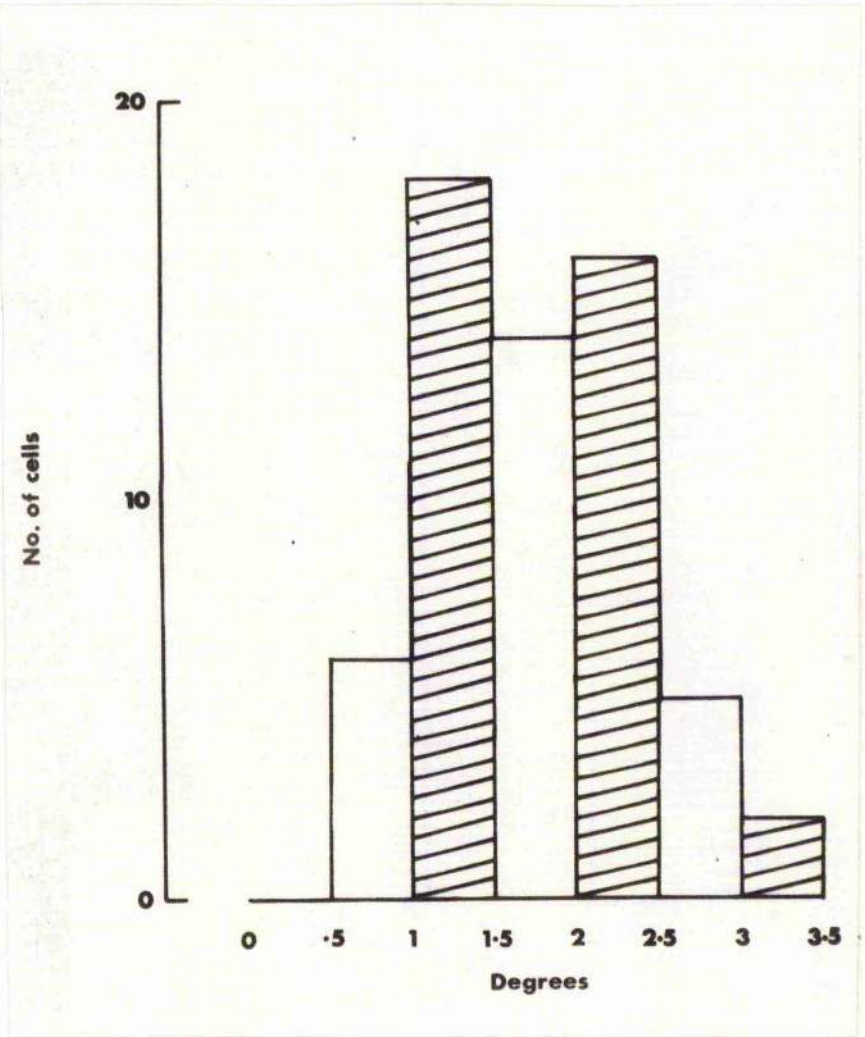


Fig. 13. Distribution of angles between the visual axes of neighbouring retinula cells. Shaded areas represent an angle equal to the inter-ommatidial angle or to small multiples of it.

D. Amplitude of the receptor potential and the angle of incidence of a stimulating light.

The amplitude of the generator potential was reduced if the light source was moved in any direction from the visual axis of the cell (sec 3:C). In a series of 15 cells the amplitude reduction was measured in both planes of the head against angular divergence of the stimulating light. This was done by measuring the horizontal visual field of the cell first in the plane of the axis and then in successive horizontal planes separated vertically by two degrees.

The average readings of the amplitude distribution in two planes about the axis of 15 cells are shown in table 2. In this table 100% (co-ordinates 0^{o}H : 0^{o}V) represents the average axis response amplitude and other values are expressed as a percentage of this.

From this data a series of contours of equiamplitude were constructed around the visual axis. Fig. 14, shows the completed diagram. Contours have been drawn at the 90, 80, 50, 30, 20 and 10% response amplitude levels, and angles of inclination are indicated by the inner and outer dotted circles which represent 5 and 10^{o} respectively from the axis. The contours form a series of ellipses around the visual axis with the longest diameter lying in the horizontal plane of the head, indicating differences in the sensitivity distribution between the horizontal and the vertical planes of the cell.

The acceptance curve of these cells was studied more closely in the absolute horizontal and vertical planes, i.e. where one of the

Table 2.

	−10	−8	−6	−4	−2	0	+2	+4	+6	+8	+10	
+10	–	–	–	–	9	9	–	–	–	–	–	+10
+8	–	8	11	12	14	15	14	12	10	–	–	+8
+6	9	12	15	17	20	20	18	17	15	12	9	+6
+4	13	17	22	26	30	33	30	25	21	16	–	+4
+2	16	22	27	39	50	66	52	38	26	21	–	+2
0	17	25	31	47	71	100	75	48	28	21	14	0
−2	15	24	30	41	52	60	53	35	25	21	–	−2
−4	12	20	23	26	30	31	27	24	21	18	10	−4
−6	–	13	18	21	22	22	21	20	15	12	6	−6
−8	–	6	11	12	16	17	15	12	10	5	2	−8
−10	–	–	–	–	–	9	–	–	–	–	–	−10
	−10	−8	−6	−4	−2	0	+2	+4	+6	+8	+10	

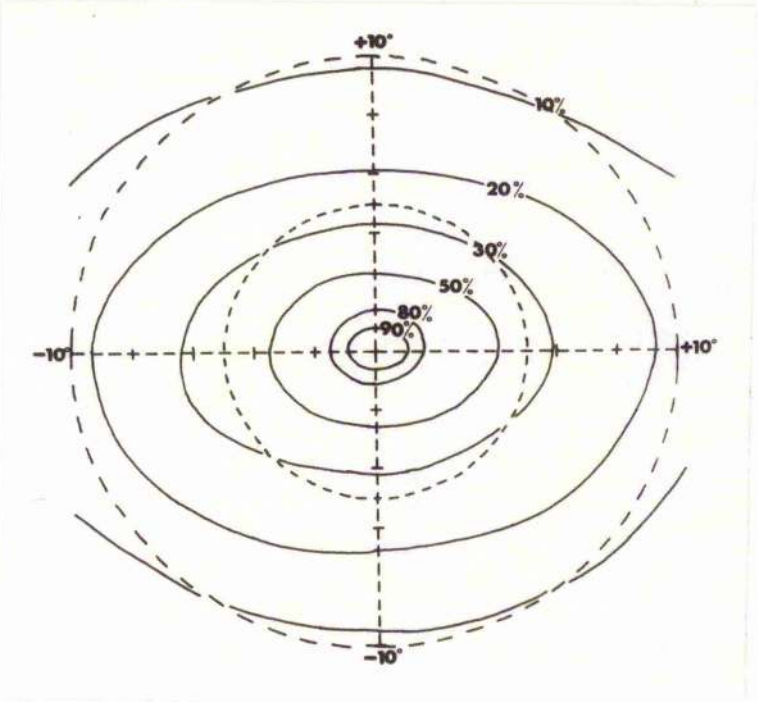


Fig. 14. Contours of equiamplitude plotted for all directions around the visual axis where an equal response was recorded. Percent responses are shown on the contours and directions of stimulus incidence are indicated by the dotted lines.

co-ordinates equals 0°. The average amplitude values along these planes with standard deviations are shown in table 3, and the mean data is drawn against angle of incidence in Fig. 13 for the horizontal plane (closed circles) and the vertical plane (open circles).

Table 3.

		H. Amp. %	V. Amp. %
Degrees from axis	+10	14 ± 6	9 ± 7
	+9		11 ± 7
	+8	21 ± 7	15 ± 5.5
	+7	24 ± 4.5	17 ± 5
	+6	28 ± 7.5	20 ± 6
	+5	37 ± 6.5	23 ± 7
	+4	48 ± 8	33 ± 6.5
	+3	60 ± 6	45 ± 8
	+2	75 ± 9	66 ± 9
	+1	92 ± 8	87 ± 10.5
Axis	0	100	100 ±
Degrees from axis	-1	90 ± 8	83 ± 6.5
	-2	71 ± 11	60 ± 9
	-3	58 ± 6	43 ± 9
	-4	47 ± 8	31 ± 6
	-5	39 ± 7	26 ± 4.5
	-6	31 ± 6	22 ± 6
	-7	28 ± 7	19 ± 8
	-8	25 ± 7	16 ± 7
	-9		13.5 ± 6.5
	-10	17 ± 8.5	9 ± 5

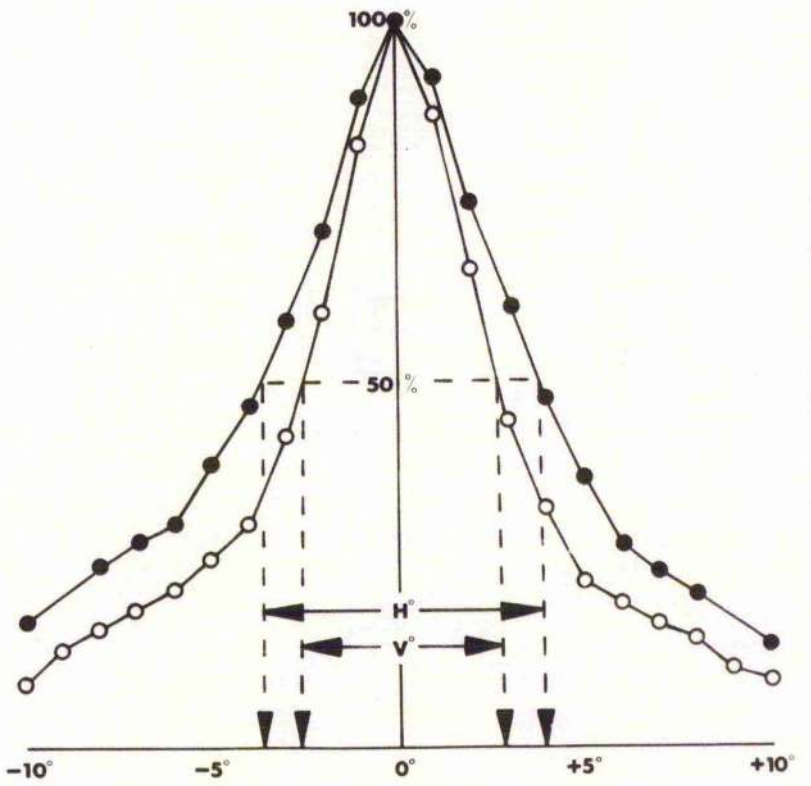


Fig. 15. Amplitude of the generator potential plotted against the angle of incidence of the stimulating light in the horizontal plane (closed circles) and the vertical plane (open circles). 0° represents the visual axis and the + and - flanks refer to the position of the light source relative to the front or top of the head. (See fig. 8).

H° and V° show the angular width of the acceptance curves at half amplitude.

The acceptance curves are more or less symmetrical in both planes of the cell, but the acceptance curve is broader in the horizontal plane. In this plane the response amplitude falls to 50% of its axis value at an angle of incidence of 3.8°. The corresponding measurement in the vertical plane shows that the response falls to the 50% amplitude level $2.6 - 2.7^{\circ}$ from the axis. The total spread of these curves at this level measures:

$$\text{Horizontal} = 7.4^{\circ}$$

$$\text{Vertical} = 5.3^{\circ}$$

Although no effort was made to control the adaptive state of the cells it is clear from this result that the visual field of a single cell is greater in the horizontal than in the vertical plane. It is possible that this difference reflects the difference between the inter-ommatidial angle in these planes (see Sec. 3K).

Although most of the measurements were taken from cells lying on or near the equator of the partial sphere through which the stimulator was moved, the absolute position of the light relative to the equator was not measured, and the correction factor (see p51) could not be used.

E. Spread of the amplitude inclination curves and the adaptive state of the cell.

The amplitude of the receptor response to light at various angles of incidence was studied in the horizontal plane of the visual field of 200 cells while the cell under observation was kept in one of the extreme conditions of adaptation. In 50 cells it was possible to

study the amplitude inclination relationship in the same cell under both conditions.

Average values of dark-adapted response amplitudes are compared in table 4 with average values of the light-adapted response amplitude from the 50 cells which were studied under both conditions. The results with standard deviations are expressed as a percentage of the axis amplitude for every degree position up to 10° to either side of the axis.

Fig. 16 drawn from the data of the table 4 illustrates relative differences between the responses of dark-adapted and light-adapted cells to a stimulating light of constant intensity.

Under both adaptive conditions the acceptance curves of the retinula cells are more or less symmetrical about the axis. In the dark-adapted cell the response falls to 50% of the axis amplitude when light is incident at 5.6° from the axis along the negative flank and at 5.4° along the positive flank, giving a total spread of 11° at this level. In the narrower light-adapted acceptance curve, the response falls to 50% at 2.7° and $+3^{\circ}$, giving a total spread of 5.7°. Because the amplitude of the generator potential is related to the intensity of the stimulating light (fig. 12) it is possible to determine the effective intensity of the stimulating light, i.e. the proportion of the light perceived by the cell, at all angles of incidence. This indicates the directional sensitivity of the cell.

The mean data of the amplitude/inclination curves was converted into terms of effective intensity using the relative amplitude-relative

Table 4

		Dark Adapted			Light Adapted	
		x%	s.%		x%	s.%
Degrees from Axis	10	20	3.6	10	8	3
	9	25	3.1	9	10	3.8
	8	30	3.3	8	12	4.3
	7	34	3.5	7	13	4.8
	6	42	3.8	6	15	5
	5	53	3.2	5	23	5.4
	4	63	3.0	4	32	5.2
	3	75	3.8	3	49	5.2
	2	85	2.5	2	66	5.7
	1	94	4.8	1	90	6.4
Axis.	0	100	0	0	100	0
	- 1	95	4.2	- 1	85	5.9
	- 2	83	3.1	- 2	60	6.2
	- 3	73	3.6	- 3	46	5.75
	- 4	62	3.4	- 4	32	5.3
	- 5	54	2.8	- 5	25	4.9
	- 6	48	2.9	- 6	19	5.1
	- 7	40	3.2	- 7	17	3.7
	- 8	35	3.4	- 8	15	4.1
	- 9	29	3.4	- 9	11	3
Degrees from Axis	-10	22	3.2	-10	6	2.1

100% = 34.3mV. n = 50 100% = 7.3mV.

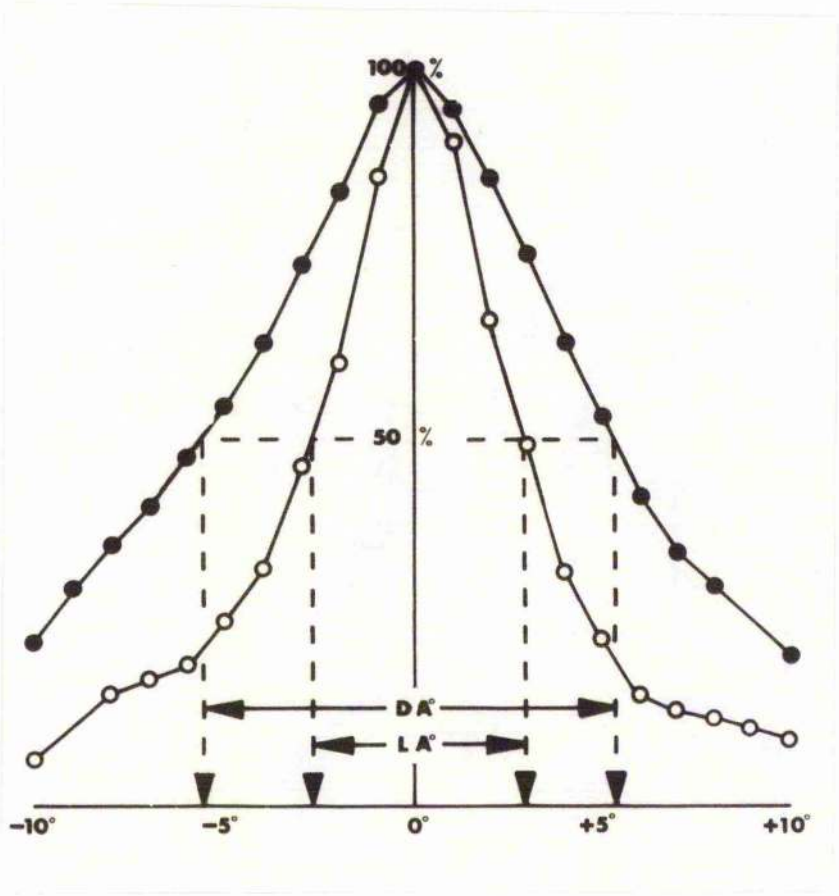


Fig. 16. Average response amplitudes of the generator potential of dark-adapted (closed circles) and light-adapted (open circles) retinula cells as a function of the angle of stimulus incidence.

100% d.a. = 34.3 mV

100% l.a. = 7.3 mV

D.A.° and L.A.° shows the spread of these curves at 50% response amplitude.

intensity curve of fig. 12. This data gives a measure of the directional sensitivity of light - and dark-adapted cells, and is plotted in fig. 17. These curves show differences in the sensitivity of retinula cells to off-axis stimulation under light- and dark-adapted conditions, e.g. when light is 2.4° from the axis (2.4° = inter-ommatidial angle) the dark-adapted cell 'sees' 60% of the intensity of the stimulating light, while the light-adapted cell 'sees' only 30% of the intensity at the same angle of incidence.

The spread of the directional sensitivity curves at 50% sensitivity is a measure of $\Delta\rho$ (see p 24) for the retinula cell. The average value of $\Delta\rho$ given by this method for dark-adapted cells equals 6.6°, varying between 6° - 7.3°. For light-adapted cells $\Delta\rho$ is smaller, with an average value of 3.4°, varying from 3° - 4.9°.

N.B. The range of $\Delta\rho$ in both instances was converted from the relative amplitude-relative intensity curve for the range within the standard deviations of the amplitude inclination curves.

In a separate series of experiments, involving 10 cells, $\Delta\rho$ was determined by an equal response method. After the visual axis of a cell had been determined, the intensity of the stimulating light was reduced to a known value with neutral density filters, and the axis response of the cell then measured. The filters were then removed and the light source moved from the axis until a position in the visual field was found where the cell gave this response. The angular distance the light had been moved is a measure of ½ of the total spread of the

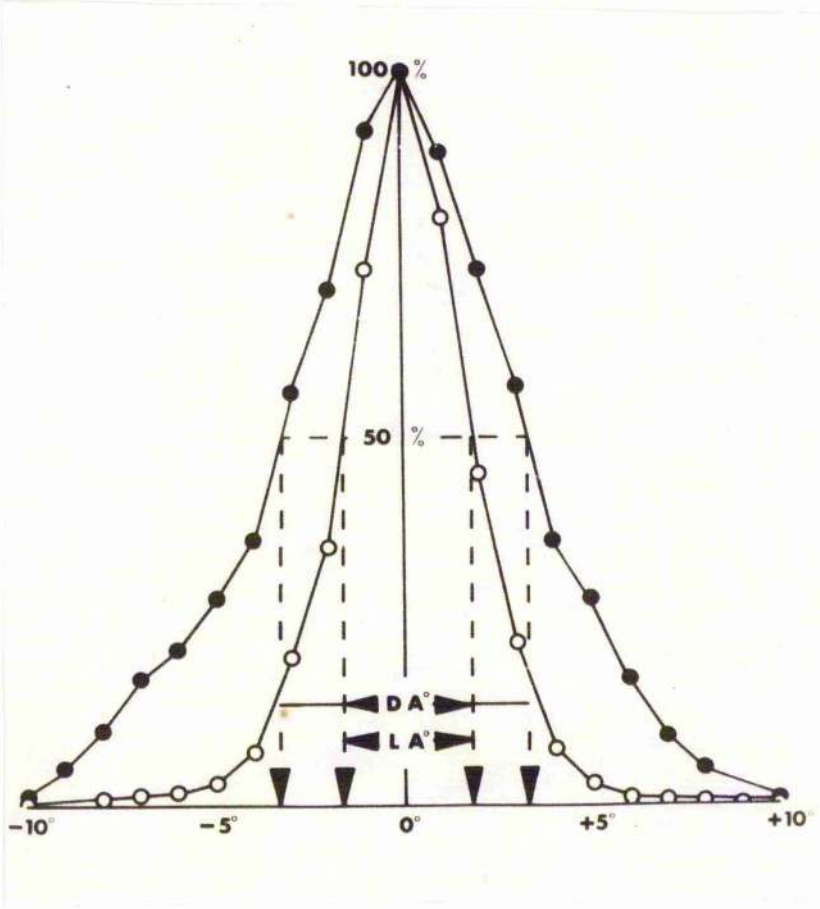


Fig. 17. The directional sensitivity curve of a dark- (closed circles) and a light-adapted (open circles) retinula cell drawn on a linear scale of sensitivity. Axis sensitivity is called 100% sensitivity for both the dark- and the light-adapted cell. These curves express quantitatively the visual field of a single receptor cell under both conditions of adaptation.

D.A.° and L.A.° indicate the angular width of the visual field of a single cell at 50% linear sensitivity ($= \Delta\rho$) under both conditions of adaptation.

directional sensitivity curve at the chosen effective intensity level.

To measure two neutral density filters which transmitted 58% and 42% of the incident intensity (see page 55) were chosen, and two points on the directional sensitivity of each cell were obtained. A straight line joining these points passed through the 50% level and a value of was obtained by interpolation.

Accuracy in this method was limited because the angle measuring device was only calibrated to the nearest 0.5°. For five light adapted cells values of ranged from 2° to 5° with a mean at 3.4° ± 0.4°. For five dark-adapted cells the mean value of was 6.2° ± 1°. These values are not significantly different from the estimates of derived from the amplitude inclination curves.

These results show that a dark-adapted retinula cell has a greater sensitivity (approx. x 2) to off-axis stimulation than a light-adapted retinula cell. These differences in off-axis sensitivity must be reflected in a relatively greater off-axis sensitivity increase than axis sensitivity increase during dark-adaptation (sec. 3.F).

F. Sensitivity changes with light and dark adaptation

1) Increase in axis sensitivity occurring during dark adaptation

Constant intensity flashes of 100 ms duration, with an interflash interval of 5–10 secs, produce responses of equal amplitude from a dark adapted cell, i.e. the cell remains in a constant state of adaptation. In the presence of an adapting light similar test flashes evoke smaller responses, which under the conditions used in these experiments were 15–20% of the dark-adapted response amplitude. The

amount by which the light intensity must be reduced, 1.5 - 2 log units, to evoke a 20% response from a dark adapted cell, is a measure of the sensitivity decrease with light adaptation.

When the adapting light is switched off, the amplitude of the cell's response to flashes of constant intensity increases with continuing time in the dark. In fig. 18 the amplitude of the receptor response is plotted against time in the dark. The response amplitude of the dark-adapted cell is indicated at the top left of the figure. With light-adaptation the amplitude is reduced, as indicated by the dashed line, to the level indicated at time zero. Test flashes were applied 5, 10, 30, 60, 120 and 180 seconds after the adapting light was turned off. The amplitude of the response recovers rapidly to 80% of the dark-adapted amplitude in the first 30 seconds of dark adaptation. The rate of recovery is slower after this time and takes approximately a further 2.5 mins to reach maximum amplitude.

Values of the corresponding increase in axis sensitivity, calculated from the energy/amplitude curve (fig. 12) are shown in table 5.

2) Increase in off-axis sensitivity occurring during dark adaptation

Because a dark-adapted retinula cell is more sensitive to off-axis light sources than a light-adapted cell (sec. 3:E), the increase in sensitivity during dark adaptation to off-axis light sources must be greater, depending upon the factors controlling the acceptance angle under the two extreme conditions, than the corresponding sensitivity change to on-axis light over the same time interval.

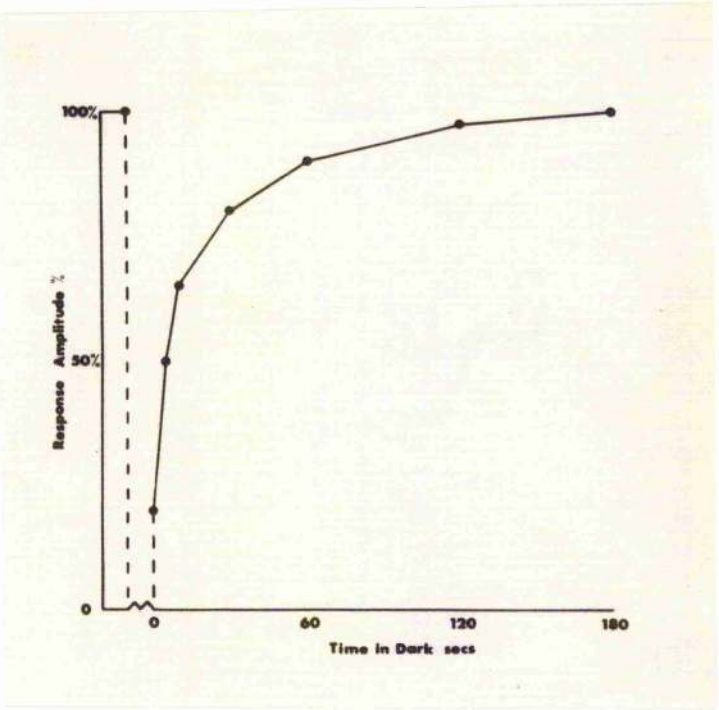


Fig. 18. Rate of axis amplitude recovery during dark-adaptation. Time zero seconds represents the end of a 10 minute period of light-adaptation. The response amplitude at this time was measured while the adapting light was still on.

In two series of experiments involving ten cells, the time course of the sensitivity increase to off-axis stimulation was studied during dark-adaptation, and was compared with the corresponding increase in axis sensitivity.

Series one

The stimulating light was moved 1.5 - 2° from the axis of a light-adapted cell until it had a stimulating efficiency of 50%I. The adapting light was then turned off and the cell allowed to dark adapt. Test flashes were applied from this angle of incidence at various time intervals and the rate of sensitivity increase studied.

Series two

In this series the stimulating light was moved 3° - 3.5° from the axis of a light adapted cell. It was calculated from the dark-adapted directional sensitivity curves that in this position it would have a 50% efficiency in the subsequently dark adapted cell. Test flashes at this inclination and at the same time intervals as in the experiments of series one were applied during dark adaptation.

The average results of these experiments are compared with the sensitivity increase on axis in table 5. Measurements are expressed as a percentage amplitude of the axis potential of the dark-adapted cell. Sensitivity (col. 2) is computed (as a percentage of axis sensitivity) from the relative amplitude - relative intensity curve (fig. 12).

In column 3 of the table sensitivity increase at various angles

of incidence is plotted as a percentage of the axis sensitivity increase. If the directional sensitivity curves did not broaden during dark adaptation Δp (light-adapted) = Δp (dark-adapted) and the increase in sensitivity would be equivalent at any position on the acceptance curve, so that sensitivity 1.5° from axis of the cell would be the same under both conditions of adaptation.

In column 3 of the table and in the figure (fig. 19) drawn from this data, the initial sensitivity of the cell to off-axis stimulation has been equated to the initial axis sensitivity. The figure shows an equivalent increase in sensitivity for all angles of incidence over the first 30 - 60 seconds of dark adaptation. After this time sensitivity to axial stimulation increases more slowly and reaches a maximum after 180 seconds. Sensitivity to off-axis stimulation, however, goes on increasing. If the total axis sensitivity increase is considered to be unity, sensitivity to sources $1.5 - 2^{\circ}$ from the axis increases by a factor of 1.4 during dark adaptation and sensitivity to lights $3^{\circ} - 3.5^{\circ}$ from the axis is more than doubled.

The data of table 5 provides three points, axis, $1.5 - 2^{\circ}$ and $3 - 3.5^{\circ}$ from the axis, on the directional sensitivity curve of a single cell at any of the stated time intervals after the beginning of dark adaptation. The changing directional sensitivity curve of a dark-adapting cell is shown in fig. 20. From this figure Δp can be measured at any time interval after the beginning of dark adaptation. Initially the directional sensitivity curve is narrow $\Delta p = 3.5^{\circ}$, but because of

Table 5

	Axis.			1.5° - 2° from Axis.			3° - 3.5° from Axis.		
Time	Amp%	Sens.%	Sens. inc.%	Amp%	Sens.%	Sens. inc.%	Amp%	Sens.%	Sens. inc.%
0	20	1.8	1.8	14	.9	1.8	10	.4	1.8
5	50	23	23	36	11.5	23	29	5.2	23.4
10	66	45	45	52	24	48	36	11	49.5
30	80	68	68	63	36.5	73	40	17.5	78.75
60	90	80	80	66	45	90	48	21.5	96.75
120	95	92	92	72	52	104	54	28.5	128.25
180	100	100	100	76	61.5	123	63	36	162.0
240	"	"	"	79	66	132	66	45	193.5
300	"	"	"	83	70	140	68	47	211.5
360	"	"	"	84	72	144	71	50	225.0

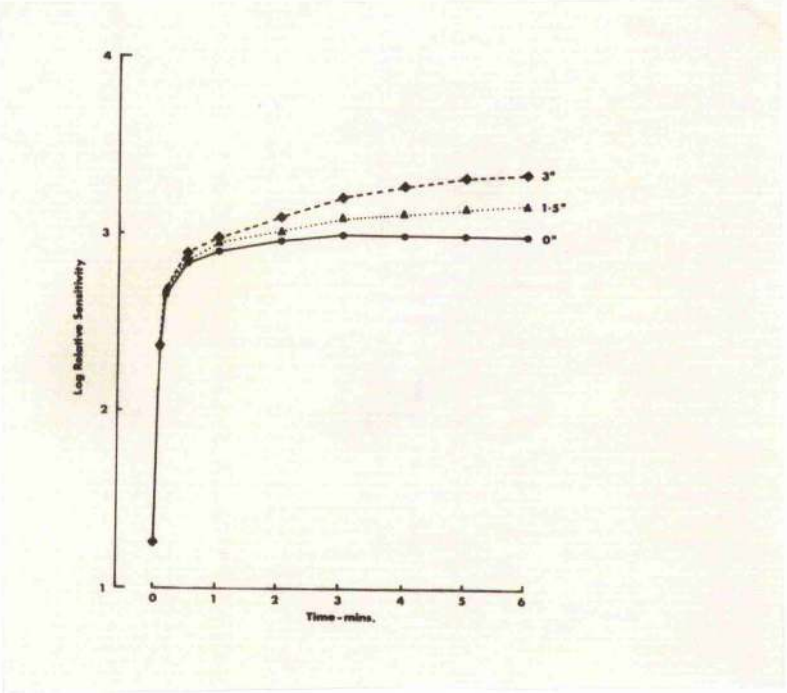


Fig. 19. Rate of increase, during dark-adaptation of a retinula cell's sensitivity to a stimulating light on its axis (solid line) 1.5° from its axis (dotted line) and 3° from its axis (dashed line). See table 5 and page 82 .

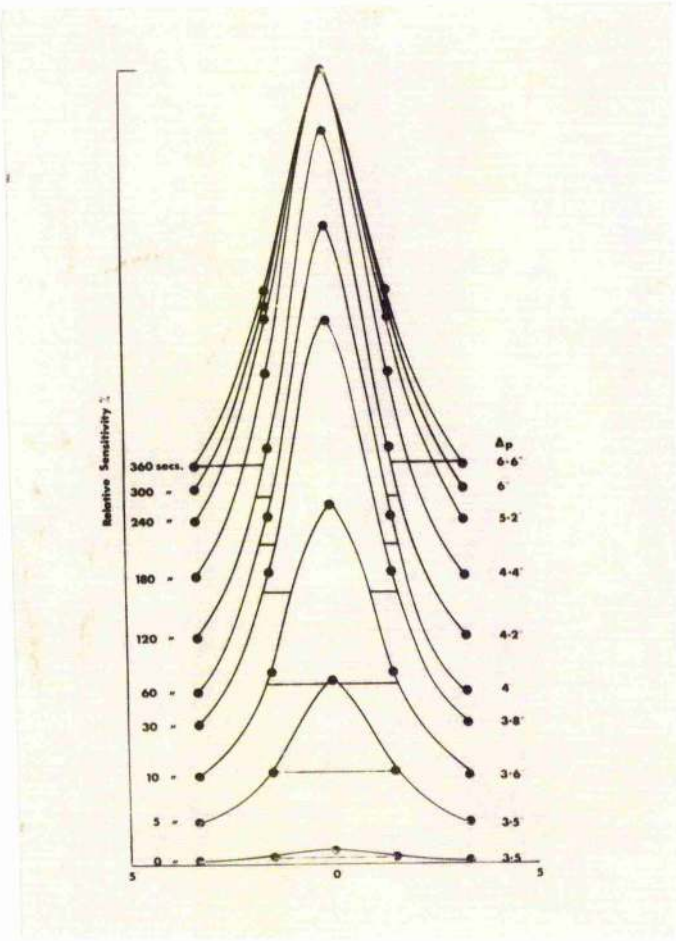


Fig. 20. Changes in the directional sensitivity curve of a single cell during dark-adaptation. The figure is drawn on a linear scale of sensitivity from the data presented in Table 5. The numbers to the left of the figure show periods of dark adaptation. Values of Δp, represented as the horizontal lines, are listed to the right. The figure shows that over the first two minutes the major change is the increase in axis sensitivity. Increase in off-axis sensitivity after this time broadens the directional sensitivity curve to the dark-adapted value.

the differential sensitivity increase during dark-adaptation becomes progressively broader. After 2 - 3 minutes in the dark axis sensitivity increase is complete and at this time $\Delta p = 4.4^{o}$, however, off-axis sensitivity goes on increasing up to 5 - 6 minutes and consequently increases up to the dark-adapted value.

The rate of increase of Δp from the light-adapted value to the larger dark-adapted value is compared with the rate of development of the palisade in section 3:J. Fig. 28.

G. Resolution of the motion of striped patterns by single retinula cells as a function of acceptance angle.

When striped patterns are moved slowly through the visual field of single retinula cells they cause a temporal fluctuation in the generator potential which is related to the spatial translocation of the stripes. These fluctuations are for a pattern of constant motion and constant λ (pattern repeat distance), dependent upon the width of the acceptance curve.

Changes in relative amplitude of the cell's response to striped patterns can be understood if it is assumed that the receptor potential is a known function of the total light flux which can be determined as the integral of the pattern light intensity, within the solid angle of the visual field. For broad stripes which fill the field, effective intensity fluctuations, and hence potential fluctuations within the cell, will be at a maximum and will follow the fluctuation of pattern contrast about the projection of its visual axis. In this case $m_e = m_a$ (see p 26)

where ma = effective intensity fluctuation within the cell, and me = pattern contrast. If stripewidth is reduced or $\Delta\rho$ increased, ma will be some fraction of available pattern contrast. (See fig. 21).

As the amplitude of the retinula cell response is not linear with light intensity, observed membrane potential fluctuations must be converted into effective intensity (eI) fluctuations. Values of this function, defined as

$$\frac{\text{eI max} - \text{eI min}}{2 \text{ average eI}} = \text{ma},$$

vary between 0 and 1 and give a direct measure of the contrast in the pattern as seen by the retinula cell. The fraction of the pattern contrast perceived by the retinula cell, $\frac{ma}{me}$, is a direct measure of the contrast transfer of the dioptric system of a single ommatidium.

Sixty degree blocks of stripes of 20, 10, 6, 4, 2, and 1° repeat distances separated by 60° totally black areas were drawn on a belt of transparent paper. The belt was driven at a constant speed (2°/sec) past a window which subtended 50° at the eye surface. A 4° white stripe, moved at each end of the window, gave no response in the retinula cell, which ensured that the visual field did not include the edge of the window. The patterns were illuminated from behind and contrast between successive black and white stripes was the same for all stripewidths; therefore pattern contrast (me) remained the same and was considered as unity in these experiments.

Fig. 22 illustrates the potential fluctuations recorded from a dark-adapted cell (A) and a partially light-adapted cell (B), when the

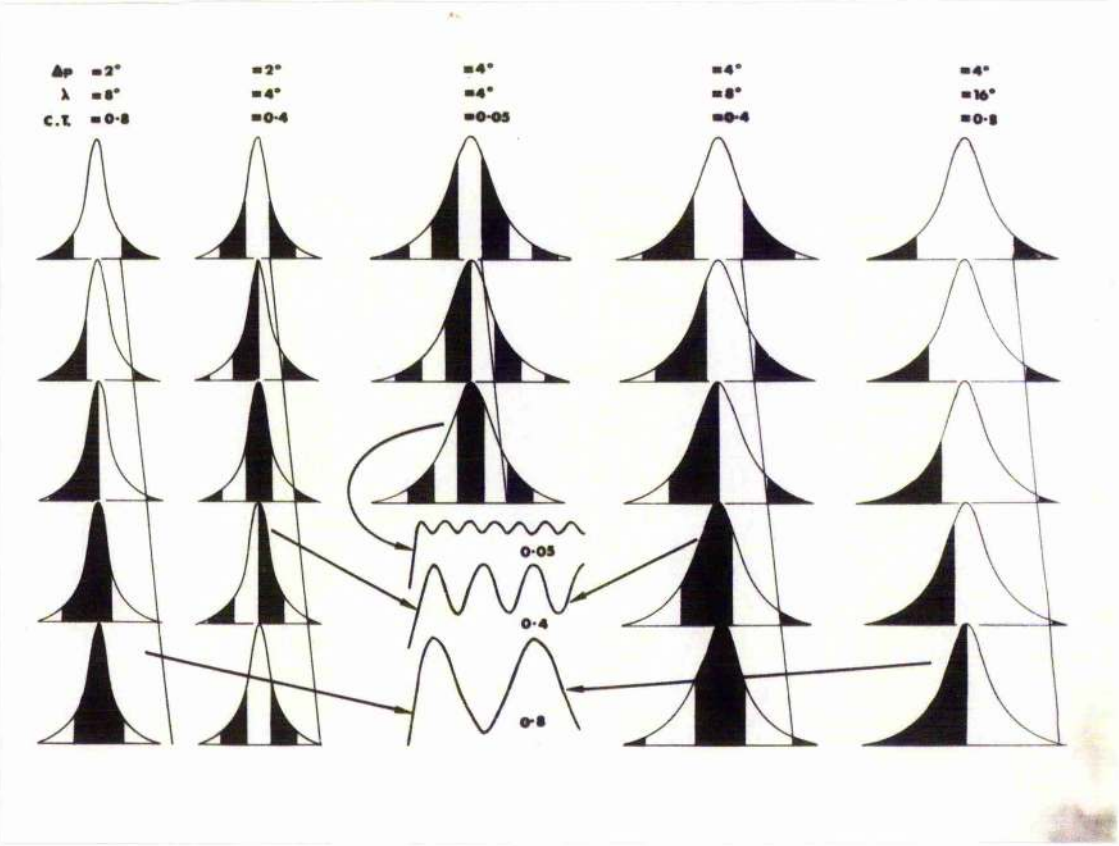


Fig. 21. Illustrates the effect of the reduction of λ or the increase of Δp upon the effective intensity fluctuation in a single cell. Effective intensity was calculated as the integral of all illuminated areas within the curve which describes the visual field of the cell. The figure shows that if Δp increases λ must also be increased to give the same effective intensity fluctuation within the cell, and hence the same contrast transfer. Effective intensity fluctuations have been calculated for various stripe widths moved by a distance (represented by the sloping line) equal to $\frac{1}{2}\lambda$, through the visual field of cells where $\Delta p = 2^{\circ}$ and $\Delta p = 4^{\circ}$.

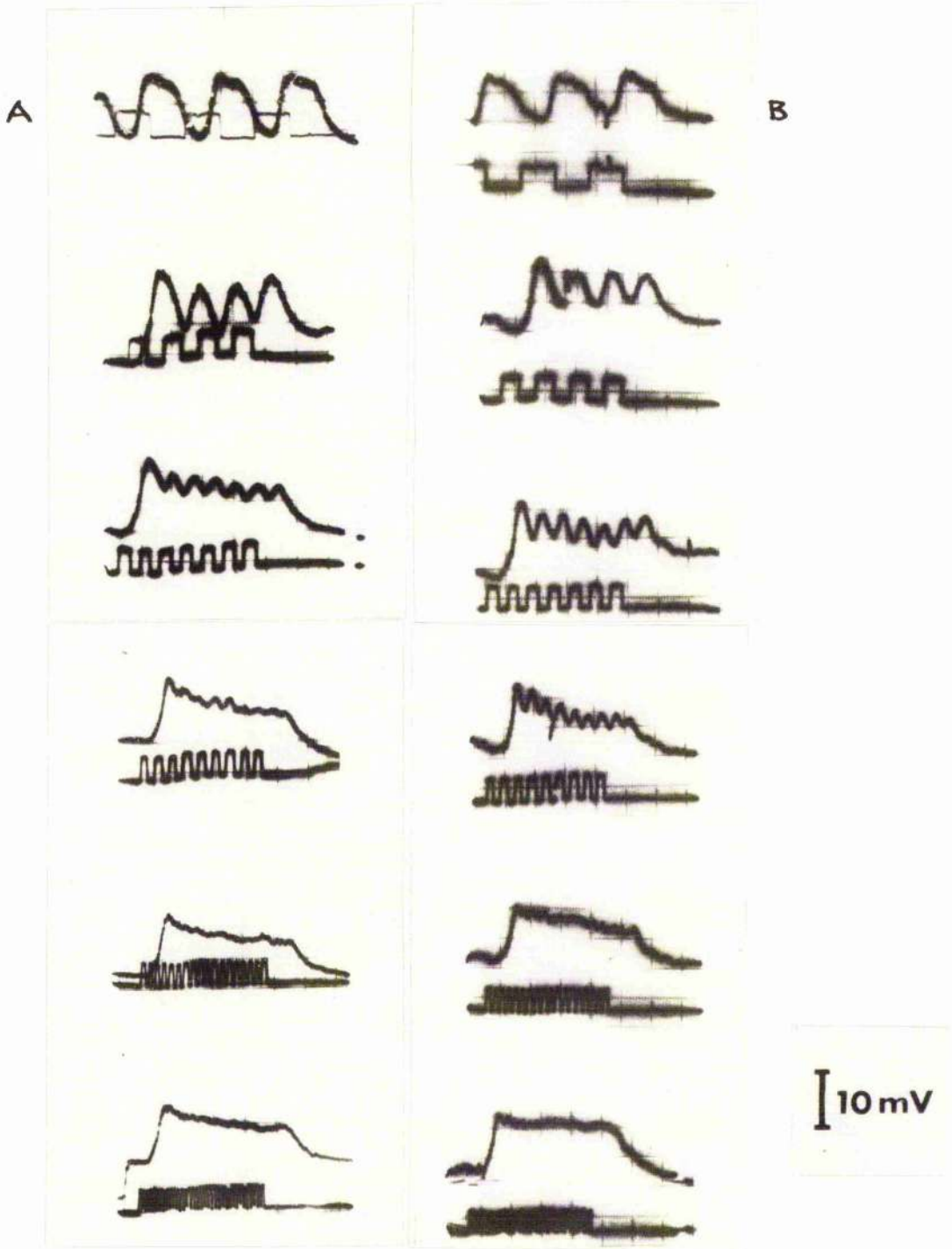


Fig. 22. The response of a single retinula cell to the movement of equal width black and white stripes of repeat distances 20°, 10°, 6°, 4° and 2° at the eye. The response of the dark-adapted cell (A) to the small stripes, is smaller than the response in the partially light-adapted cell (B), because of the larger Δp in the dark-adapted cell.

stripes moved through their visual field. The lower trace in each frame is the response of a photocell to this motion. The two traces do not coincide in time because the photocell and the retinula cell, both with restricted apertures, are looking at different areas of the total 30° stripe field. For a pattern of constant motion the amplitude difference between the maxima and the minima is progressively reduced with reduction in stripe width (successive frames in fig. 22) or with increase in $\Delta\rho$. These potential fluctuations were converted into effective intensity fluctuations and ma wms calculated. The fraction, $\frac{ma}{me}$ = contrast transfer, is plotted against λ in Fig 23 and is compared with theoretical values derived from the expression of contrast transfer, for Gaussian curves of $\Delta\rho = 2^{\circ} - 6.5^{\circ}$. In the figure open circles represent light-adapted cells and closed circles, dark-adapted cells. Horizontal bars indicate the total spread of points at each value of λ for 19 cells.

The average experimental curves agree with theory based upon Gaussian acceptance angle curves of $\Delta\rho = 5.4^{\circ}$ and 3.8° for dark and light-adapted cells respectively, over the range $\lambda = 5^{\circ} - 15^{\circ}$. The resolution of smaller stripe widths is, however, greater than predicted. Theoretically when $\Delta\rho = \lambda$ contrast transfer should fall to 0.027; i.e. there should be a 2.7% intensity shift from maximum to minimum. However, experimental values greater than 5% are recorded. This is probably because the actual acceptance curve of the receptor cell is sharper than a Gaussian curve with the same $\Delta\rho$.

H. <u>The response of single cells to sinusoidally oscillated point sources</u>

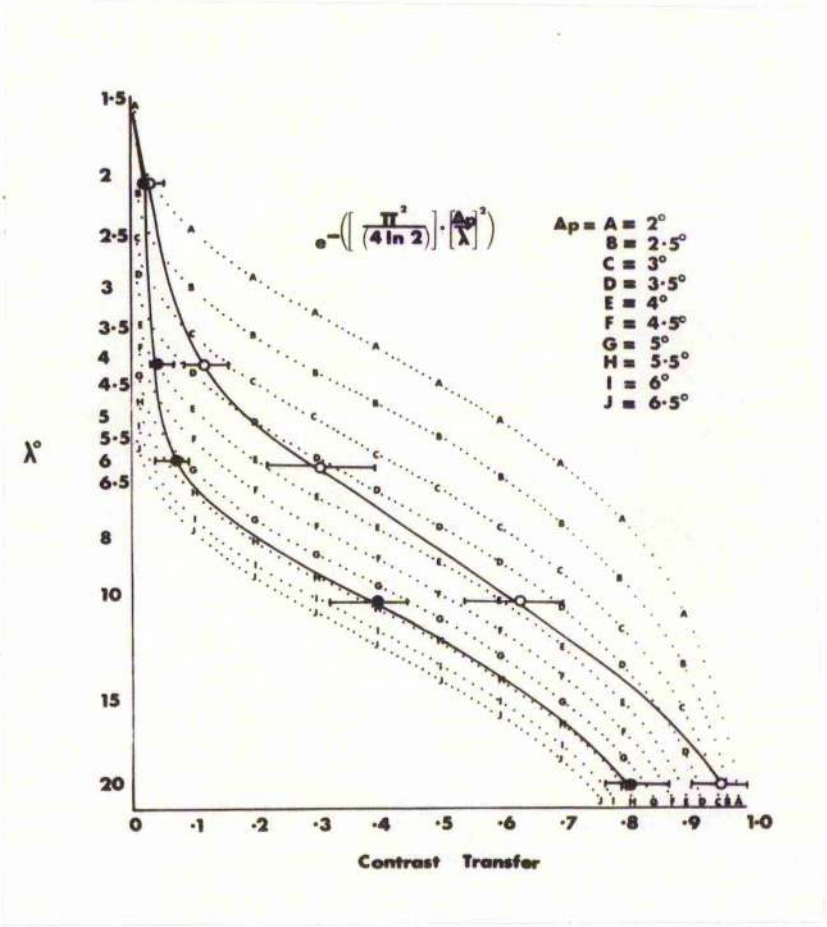


Fig. 23. Experimental curves (solid lines) of contrast between the maxima and minima of the retinula cell's response to the movement of stripes of various pattern repeat distance (λ). Open circles represent light-adapted cells and closed circles represent dark-adapted cells, the horizontal bars indicate the scatter of observations. These curves are compared with theoretical curves (dotted lines) computed from the function of contrast transfer for various values of Δp, and show how contrast between stripes as seen by the cell increases with increasing λ and does so more quickly for cells with narrower directional sensitivity curves.

Sensitivity of the retinula cell to the movements of small lights was studied by sinusoidally oscillating a point source in a plane through their visual field.

It was also possible with this method to observe the response amplitude distribution about the axis although the adaptive state of the cell changes slightly during the stimulus presentation time.

In figure 24 the amplitude of the oscillation was increased from 2° to 4°, then by 4° steps to 28°, while the mid point of the oscillation remained on the axis i.e. the light moved 1°, 2°, 4°, 6° etc. up to 14° to either side of the axis of the cell. Since the oscillating light source passed through the axis of the cell twice in one cycle, the peak amplitude is observed twice per cycle.

Values of amplitude of the potential for various angles of incidence, derived from this figure, are tabulated below.

Angle from axis	Amplitude %
0°	100%
1°	85
2°	62
4°	40
6°	15
8°	5
10°	0
12°	0
14°	0

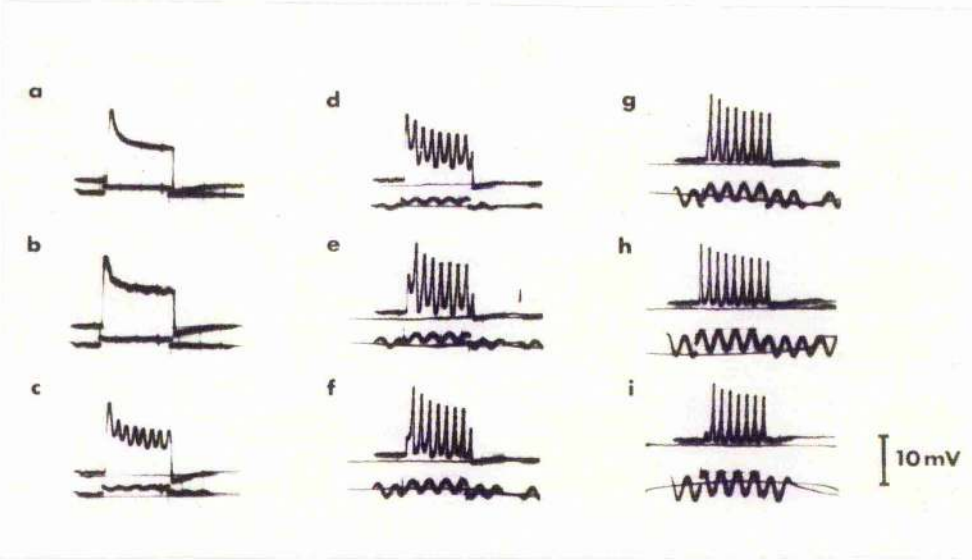


Fig. 24. Response of a retinula cell to a point source of light sinusoidally oscillated in a plane about its axis. a) is the response of the cell to the stationary light, in b) the light moves 1° to either side of the axis. c) - i) the amplitude of the movement is increased from 2° up to a 14° movement to either side of the axis.

The amplitude of the receptor potential is much smaller at high angles of incidence than is indicated by other methods, but the values over the range 0° - 6° agree fairly well with the amplitude inclination curve of the light-adapted cell (Fig. 16), and amplitude/incidence curves drawn for this data have a spread of 6° at the 50% amplitude level.

In fig. 25 the point source was oscillated with a constant 8° amplitude in the visual field of a cell and was moved in 2° steps from the axis of the cell (e in the fig), so that in c the extremes of the oscillation are at axis and at - 8°. The figure shows that the potential fluctuations in the cell's response to the movement of a point source about the axis has double the frequency but only half the amplitude of the same response to a source 4° from axis.

It may be possible to deduce from this figure what the response of the immediate neighbours of an ommatidium might be when a point source moves in its field. For example, one can assume that frames d and f represent two cells both separated by two degrees from another cell (e) which is being stimulated by a point source moving about its axis. Similarly frames c and g will represent a further two cells separated by one from the stimulated cell, and so on.

I. Inhibition in retinal receptors

There is some evidence for a light-induced inhibitory mechanism in the retina. Retinula cells which give good responses to axial stimulation can be made to show re-polarising responses to a second high intensity, off-axis source, fig. 26 or hyperpolarising potentials to

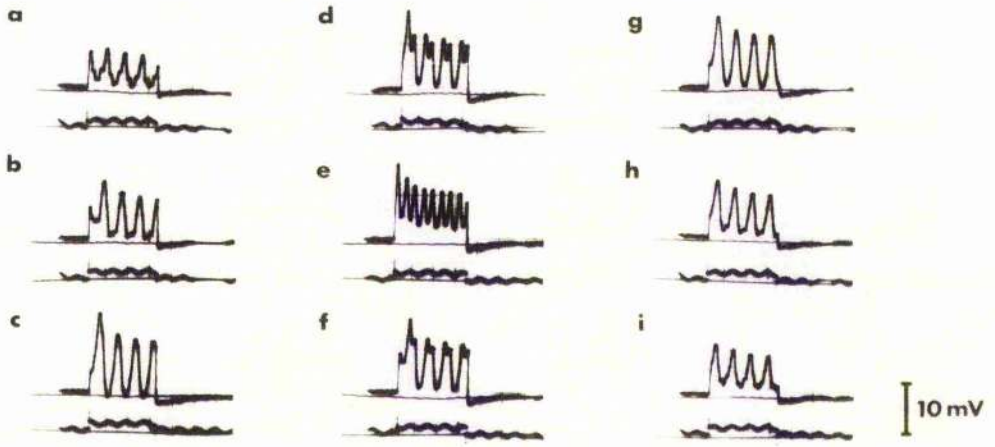


Fig. 25. Response of a retimuln cell to a point source oscillated at constant amplitude, 8°, in various parts of its acceptance field. e) shows the response when the mid-point of the oscillation is on the axis of the cell. Other responses are recorded when the mid-points of the oscillation is moved by two degrees steps in the negative horizontal flank of the visual field d - a, and in the positive horizontal flank f - i. See text page 94.

single light stimuli at large angles of incidence. The amplitude of these potentials is small relative to the positive-going intracellular potential response to axial stimulation, and is also small relative to the local E.R.G. response to the same stimulus intensity. It may therefore represent the mass response of the eye superimposed, by electronic spread, upon the membrane potential of the observed cell (Burkhardt and Autrum 1960).

On the other hand, such responses may indicate a process of simultaneous contrast enhancement similar to the lateral inhibitory system in the Limulus eye. But before accepting such an hypothesis it would have to be shown that the polarising response is active in modulating the frequency of the action potential discharge in the retinular cell axons, and as spikes are only rarely recorded in the receptor cells, this has not conclusively been shown. Scholes (1965 a,b) reports two occasions, however, where the spike discharge of a retinula cell in the dark was inhibited by light stimulation and transiently accelerated at stimulus "off", typical of inhibitory rebound. Scholes speculates, (1965b) from the similarity between this response and the response of second order cells in the dragonfly ocellus (Ruck 1961 b), that these hyperpolarising responses represent the responses of eccentric cells.

If neighbouring elements in the retina have an inhibitory effect upon the generator potential of single cells, very acute directional sensitivity curves might be expected if the visual field of the cell

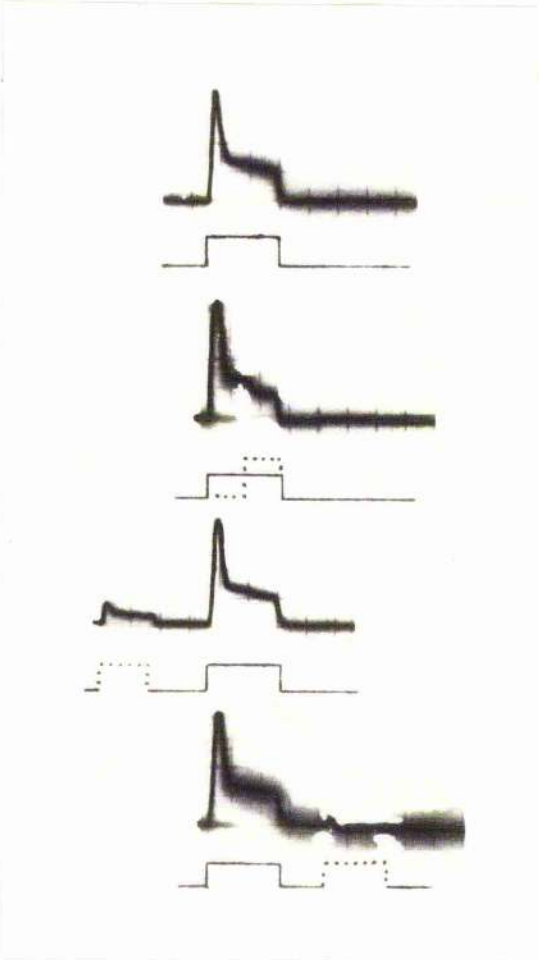


Fig. 26. Effect of a second high intensity off-axis light source on the intracellular response to axis stimulation. The axis stimulation is marked by the solid line in the stimulus monitor trace, and the second source by the dotted line.

The second light depolarises the cell if the stimulus is applied before axis stimulation. If off-axis stimulation is applied during or after axis stimulation it has a repolarising or slight hyperpolarising effect. (Retouched).

is studied in the presence of a second light, and may account for the reduction of Δp with light-adaptation.

To test this possibility two lights of equal intensity, each subtending 0.5° at the eye surface, were mounted on the points of a pair of dividers so that they could be moved apart by a known equal, angular distance from the axis of the cell. Axis was found by stimulating the cell with one of these sources, and the amplitude of the recorded potential noted. The lights were then pulsed together and separated by two degree steps. The amplitude of the response of five light-adapted and five dark-adapted cells to stimulation by both lights was measured at each position.

Fig. 27 illustrates the results from these experiments. Intensity is plotted against 2x the angle of incidence, and 50% intensity, represented by the horizontal, is equal to the intensity of a single source. Where the curves joining the points cross the horizontal dashed line each light is contributing 50% of its intensity to the total perceived by the cell, and the angle between the lights (= 2 x the angle of incidence of a single light source) should be equal to Δp. Sensitivity at the other points is derived from the amplitude - energy curve. Closed circles represent dark-adapted cells, and open circles light-adapted cells. Vertical bars show the scatter of points and the curves are drawn through the mean of the readings.

Values of Δp measured in this way vary from $2.8 - 4^{\circ}$ in light adapted cells and up to 6.4° in dark adapted retinula cells. These

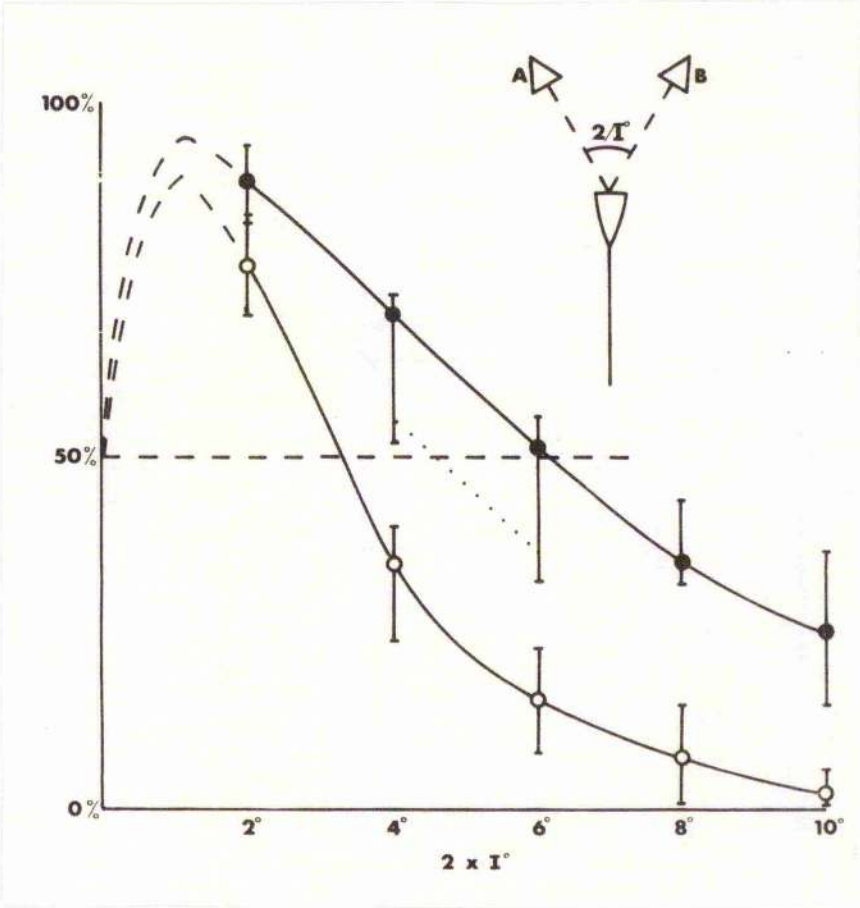


Fig. 27. Results of two light experiments. The lights A and B are moved an equal distance to either side of the retinula cell axis. The angular distance between them is then equal to 2x the angle of incidence of a single light. Where the curves cross the horizontal dotted line at 50% intensity is a measure of Δp. Closed circles represent dark-adapted cells, open circles light-adapted cells. The vertical bars show the scatter of points. (See text page 98).

values are not significantly smaller than those measured with the equal response method (page 76) or computed from the amplitude inclination curves.

It can be seen, from this data that the inhibitory influence of neighbouring cells is negligible, under the conditions used in these experiments, and cannot account for the reduction of Δp in the presence of an adapting light.

J. The development of the palisade during dark-adaptation

If the increase in Δp during dark-adaptation is due to a change in the rhabdom/cytoplasm refractive index relationship associated with the development of the palisade, the rate of increase of Δp should be correlated with the rate of development of the palisade. In order to test this possibility histological preparations of locust eyes, dark adapted for various periods, were made and anatomical changes associated with dark adaptation were studied. Fully light adapted locusts were placed in dim red light and their eyes were allowed to dark adapt. After various intervals in the dark, eye slices were cut and fixed in the dark in osmic acid (see sec.2F). After dehydrating and embedding, thin sections were cut through the distal ends of the ommatidia, and the narrowest diameter of the rhabdom and surrounding palisade was measured with a calibrated micrometer eyepiece. The narrowest diameter was measured so that obliquely cut sections would not influence the results.

In the first instance sections were taken from eyes after every 15 minutes of dark adaptation up to one hour; however no differences in the palisade plus rhabdom diameter were observed after the first 15 minutes of dark adaptation. Sections were then cut after five minutes, and finally after one minute intervals of dark adaptation.

The preparations showed variations of up to 25% at any one time and each measurement of palisade rhabdom diameter listed in table 7 is an average of readings from 5 ommatidia taken from 2 preparations and from 2 slides for each preparation.

The development of the palisade is due solely to the radial migration of mitochondria in the retinula cells. No changes in the position of pigment granules in either the retinula or primary iris cells was observed, nor were changes in the diameter of the rhabdom observed although carefully looked for. Increase during dark-adaptation of the clear area around the rhabdom, which as measured in these experiments included the rhabdom, must then represent the development of the palisade with time. By simple subtraction and division the actual width of the palisade can be calculated. The results of this study are shown in table 7 and are plotted against time in figure 28 (solid line).

The figure also shows the rate of Δ p increase with dark adaptation dashed line (see sec. 3:F. fig. 23). The curves have been made to coincide at 6 minutes i.e. at the completion of the Δ p change. The figure shows that increase in Δ p roughly parallels the development of the palisade up to 6 minutes after the beginning of dark adaptation.

Table 7.

Time in dark Mins.	Div. of Micrometer	Total Diameter	Width of Palisade
0	4.0	1.25μ	0 μ
1	4.3	1.4μ	0.075μ
2	5.3	1.73μ	0.24μ
3	7.0	2.2μ	0.475μ
4	8.3	2.7μ	0.725μ
5	10.0	3.1μ	0.925μ
10	12.5	4.0μ	1.37μ
15	14.5	4.5μ	1.625μ

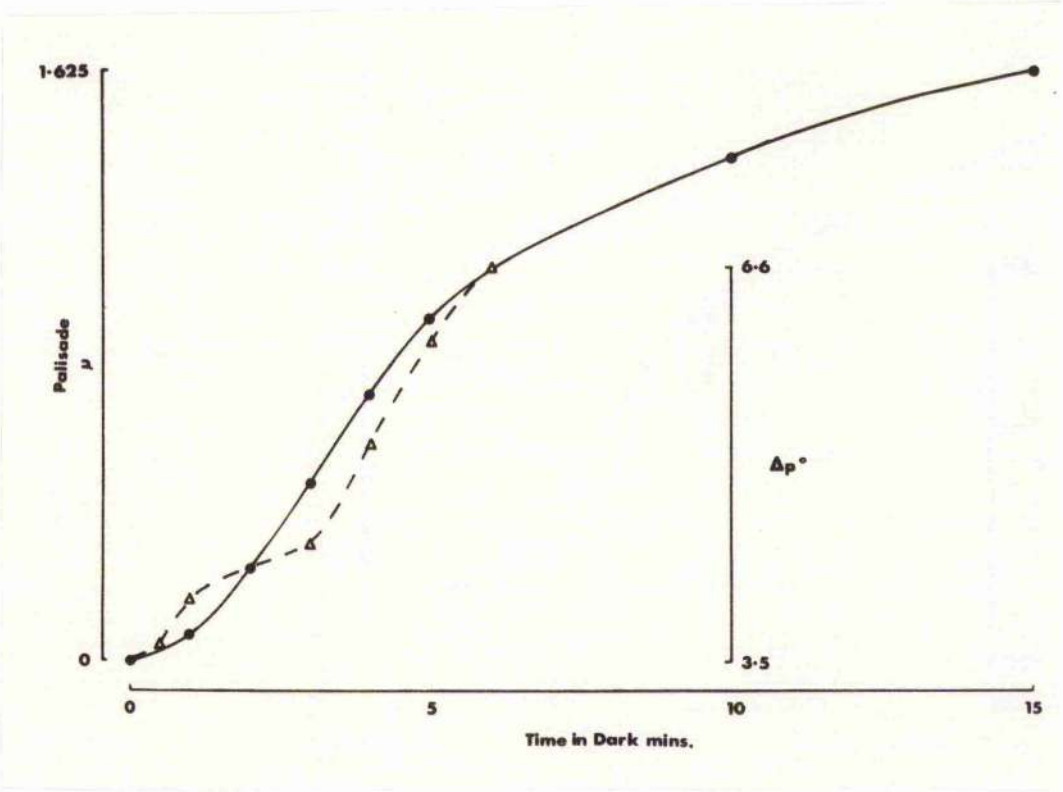


Fig. 28. The rate of development of the palisade (solid line) is compared with the rate of Δp increase (dashed line) during dark-adaptation. Values of Δp are taken from fig. 20.

Although the palisade goes on increasing after this time it has no apparent effect upon the visual field of the cell.

K. Inter-ommatidial angle and the dimensions of the ommatidium

The inter-ommatidial angle Δ° was measured in the region of the eye where electrical responses were recorded. Sections were cut in the horizontal and vertical planes so that they passed down the axes of all the cells in that region. Values of the mean inter-ommatidial angle were calculated from microphotographs of these sections (see fig. 29) by constructing lines passing down the axis of the cones at 90° to the distal cone surface and measuring the angle between them. The angle between five ommatidial pairs was measured from photographs of three different preparations.

Mean values of Δ° obtained in this way, $2.3^{\circ} \pm 0.2^{\circ}$ in the horizontal plane and $1.0^{\circ} \pm 0.3^{\circ}$ in the vertical plane, are in agreement with measurements of inter-ommatidial angle made by the pseudopupil method (Burtt and Catton 1954).

The dimensions and aperture of the crystalline cones and the dimensions of the ommatidia in the central region of the eye, were also studied from these photographs. The result of this study are tabulated below (table 8)

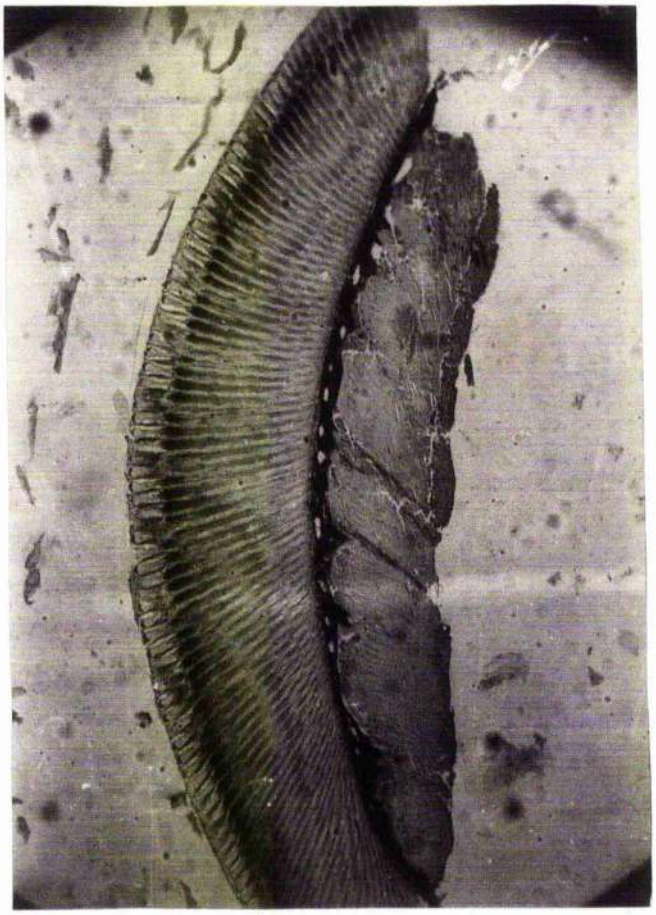


Fig. 29. Vertical section passing down the axes of the ommatidia. The cornea has been lost and slight shrinkage of the crystalline cones has occurred during the histological preparation.

Table 8.

Plane	ϕ°	Crystalline Cone			Ommatidium	
		a.	b.	d.	b.	d.
Horizontal	$2.5^{\circ} \pm 0.2^{\circ}$	$19^{\circ} \pm 0.5^{\circ}$	60-90μ	30μ	4.25μ ± 20μ	24μ ± 3μ
Vertical	$1.0^{\circ} \pm 0.3^{\circ}$	"	"	"	"	"

ϕ° = inter-ommatidial angle.

a = aperture

b = length

d = diameter

L. Refractive index measurements.

Attempts were made to measure the refractive index relationship between the rhabdom and its immediate environment in frozen sections of dark- and light-adapted locust ommatidia.

Under the interference microscope the phase retardations produced, in a beam of polarised monochromatic light, by the rhabdom and surrounding mitochondria in the light-adapted ommatidium were almost identical, and refractive index differences could not be resolved. In the dark-adapted ommatidium the palisade shows up as a bright zone between the cytoplasm and the rhabdom.

Because the thickness of the sections was unknown it was impossible to make accurate measurements of the refractive index of the important areas, however, it is clear that the refractive index change is greater between the rhabdom and its environment in the dark-adapted state.

Barnard (1964) gives phase retardations produced by the palisade of 120 - 123° and by the rhabdom of 14° - 150°. If it is assumed that the palisade consists of body-fluid, its refractive index can be readily measured. The R.I. of the body-fluid has a value of 1.348 as measured in an Abbe refractometer.

If a phase retardation of 120° corresponds to a refractive index of 1.348 the refractive index of the rhabdom can be estimated at 1.39 - 1.40. This value seems a reasonable estimate as it is comparable to refractive index measurements of the outer segments of vertebrate rods (see page 30) which have a similar membraneous structure as the insect rhabdom (Barer 1957).

4. DISCUSSION

Insofar as the intracellular response of single retinula cells in the locust eye to light at various angles of incidence gives an indication of the optical properties of the compound eye it can be stated that:

(a) the visual fields of neighbouring retinula cells overlap, and the extent of the overlap, expressed as $\frac{\Delta p^{\circ}}{\rho^{\circ}}$, increases with dark adaptation from 1.36 for light-adapted cells to 2.64 for dark-adapted cells. This is consistent with values of this ratio in other insects (table 1).

(b) retinula cell sensitivity is related to some extent to inter-ommatidial angle (sec. 3:D).

(c) the visual field of single cells must depend physically upon the geometry and refractive index of the dioptic system, and to the ratio of the refractive index in the rhabdom to that of the surrounding retinula cell cytoplasm (see below).

Measurements of the visual field of single cells show that the distribution of sensitivity about the axis of a cell, expressed as Δp, is smaller in light-adapted ($\Delta p = 3.4^{\circ}$) than in dark-adapted cells ($\Delta P = 6.6^{\circ}$). The time course of the increase in Δp during dark adaptation is correlated with the replacement of a dense layer of mitochondria, which in the light-adapted eye closely invest the rhabdom, by a fluid-filled palisade of low refractive index. It is not correlated with the more rapid increase in sensitivity of the receptors which also occurs during dark adaptation (page 78).

The critical angle for internal reflection in the rhabdom can be calculated from estimates of the refractive index of the rhabdom and mitochondria in the light-adapted ommatidium based upon refractive index measurements of the analogous structures in vertebrate rods and cones (Barer 1957) and of the rhabdom and palisade in the dark-adapted ommatidium. Justification for the following values is given after each one.

Rhabdom	$n = 1.4$	This is based on the similarity to the outer segments of vertebrate rods and cones.
Mitochondria	n_1 l.a. = 1.39.	This is a reasonable value for mitochondria in living cells and in isolated form from tissue homogenates.
Palisade	n_1 d.a. = 1.35.	This assumes that the contents of the palisade are not very different from haemolymph.

Calculation from these values shows that the development of the palisade will reduce the critical angle for internal reflection in the rhabdom from 81° in light-adapted ommatidia to 75° in dark-adapted ommatidia. These calculations indicate that the rhabdom of a light-adapted ommatidium will select by internal reflection, and transmit without refractive loss rays incident between 0° - 9° from its axis. Upon dark-adaptation the acceptance angle of the rhabdom will be broader and it will now internally reflect rays up to 15° from its axis (fig. 30).

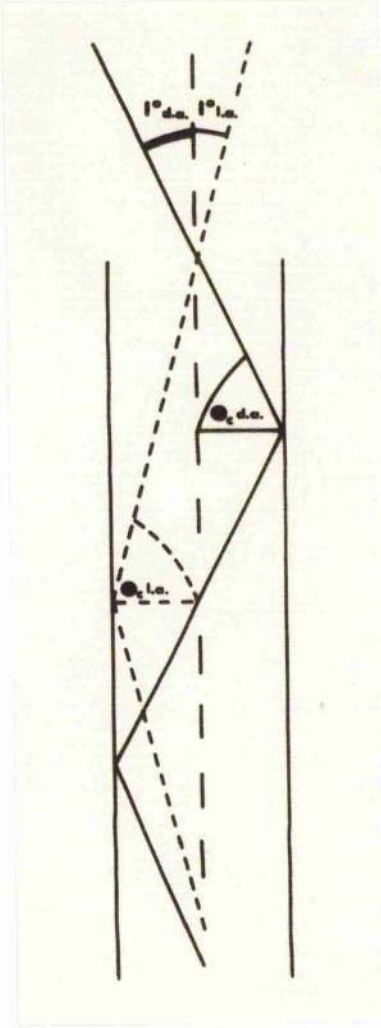


Fig. 30. The effect of a reduction of the critical angle for total internal reflection (θc) upon the acceptance angle of the rhabdom (I°).

Rays incident at the rhabdom/cytoplasm interface in the dark-adapted and light-adapted condition are represented by the solid and small dashed lines respectively. Angles of incidence are measured from the axis (large dashed line) of the rhabdom.

Subscripts d.a. and l.a. indicated dark-adapted and light-adapted.

The ratio of the calculated acceptance angle of the light-adapted to that of the dark-adapted rhabdom (I d.a. : I l.a. = 1.5) is similar to the measured ratio Δ p d.a. : Δ p l.a. = 1.9. This suggests that the change in acceptance angle, and the acceptance angle itself, depend physically upon the limits set by internal reflection in the rhabdom. This in turn suggests that the acceptance angle of the purely optical path in the cuticle and cone is wider than that in the rhabdom. This limitation of the angle of acceptance by the refractive index difference at the surface of the rhabdom must certainly hold in light-adapted conditions.

In these calculations, however, several factors which have an effect upon the acceptance angle have been disregarded. The following are assumed to remain constant during dark-adaptation and therefore to have no effect on the ratio I d.a. / I l.a.

First, the optical pathway in the ommatidium is much more complex than assumed above, and includes interfaces between air/cuticle, cuticle/cone and cone/rhabdom all of which will widen the apparent acceptance angle of the rhabdom as measured outside the animal because they refract off-axis rays incident at the facet surface.

Second, the cone as seen in histological section is a tapered structure and therefore increases the divergence of a non-axial ray which is internally reflected at its surface (see fig. 2). Because of its taper the cone will narrow the angle of acceptance of the ommatidium by reducing the angle of incidence at which a ray can strike the facet

surface and still be greater than or equal to the critical angle for reflection at the rhabdom/cytoplasm boundary.

Third, sections of the retina show that the ommatidia are not necessarily straight. The observed curvature might be an artefact, but there is no evidence that the anatomical and physiological axes of the ommatidium, or the axes of the cone and rhabdom, coincide.

No pigment migrations or increase in rhabdom diameter or other morphological changes which might increase the visual field of the ommatidium during dark adaptation have been observed in locust eyes. Therefore, in the absence of other known causes, the correlation between the increase in Δ p and the development of a palisade of low refractive index is evidence for internal reflection in the rhabdom. Based upon this, a hypothesis can be advanced that internal reflection in the rhabdom governs the visual field of the ommatidium of all compound eyes. This hypothesis is based upon:

1. The correlation in time between the development of the palisade and the increase in Δ p during dark-adaptation.

2. The ratio of the angle of incidence, at the facet surface, of light rays which are totally reflected in dark-and-light-adapted rhabdoms (I d.a. / I l.a.) is approximately equal to the ratio Δp d.a./ Δp l.a.

Such an hypothesis would explain the shape of the acceptance angle curves in pigmentless Calliphora eyes. These curves are the same as those for cells in normal eyes except that they are extended laterally.

The central region is not changed by the pigment (Washizu, Burkhardt, and Streck, 1964). The acuity to stripes is less in fly eyes which have no pigment (McCann and MacGinitie 1964) but the difference can be accounted for by the lateral spread of the curve in the pigmentless forms.

The typical acceptance curve of single cells is approximately symmetrical. However, a small number of cells have markedly a symmetrical or multiple-peaked acceptance curves which indicate anatomical asymmetry in the ommatidium. We can account for such curves most readily by supposing that:

(a) The facet is flattened or dimpled and therefore the acceptance angle of any cell in that ommatidium will have a double peaked curve. An obstruction on axis within the dioptric system would have the same effect.

or (b) The retinula cells may differ in size or in proportion of the rhabdom which each contributes.

Asymmetrical or multiple-peaked curves cannot, on the other hand, indicate a physiological peculiarity in some cells. For example, two or more cells in the ommatidium may interact electrically but the observed visual field will be a composite one of two similar visual fields if light does not keep to individual rhabdomeres. Even if some records are from eccentric cells, which presumably have an input from more than one of the retinula cells in the ommatidium, we suppose that the different retinula cells of one optically symmetrical ommatidium

all have the same acceptance angle curve.

Again, multiple-peaked acceptance curves may result from the cell's response to second and third-order diffraction images. However, if this was the case it would be reasonable to expect all cells to show multiple-peaked acceptance curves, and that there would be some correlation between the inter-ommatidial angle and the angular distance separating the peaks.

In fact, no evidence has been found to suggest diffraction phenomena which have any effect upon the retinula cell response, and if the light is not restricted to individual rhabdomeres, there is no means of inferring electrical interaction in the experiments reported here.

In broad agreement with calculations of the optical properties of compound eyes from optomotor responses (Götz 1964, 1965) the resolution of retinula cells, measured directly, shows that the minimum stripe width which the cell can resolve is related to its acceptance angle (sec 3:G). It is possible, however, that the insect is able to average tiny potential changes in many retinula cells, not evident above the recording noise level of these experiments, and have a greater resolution in the retina than these experiments suggest. This would be less feasible if the retinula cell axons transmit by spikes. It seems unlikely that individual retinula cells are able to resolve patterns of 0.3° or that responses to these patterns would be recorded from the ventral cord since some primary resolution must be lost by neural processing in the ganglia. The high resolution found by Burtt and Catton must have some other explanation, which could be that which Palka gives or which might

result from the movement of an imprecisely made pattern, giving the same effect as a single point source moving in the visual field (see below).

A high sensitivity of the eye to the movement of point sources or of clearly resolved edges (Horridge and Sandeman 1964; Thorson 1965) can be inferred from the directional sensitivity curves of single cells. If a point source is moved 0.5° from the axis of a cell (cell a, b, c. fig 31), the effective intensity in that cell will fall by some 5%. In neighbouring ommatidia on one side, however, effective intensity will increase by 15 - 20%, (cell c) if the motion of the light is toward the axis, or fall by 2 - 3% (cell a) if it is away from their axis. There is every reason to suppose that the threshold of the whole animal will be governed by those cells which are in the most sensitive part of their range for any position of a point source. By observation of the threshold of the Burtt and Catton fibre in the ventral cord Palka (1965) finds a threshold $\Delta I/I$ of 2-4% depending on I. Therefore, approximately threshold to movement of a point source will be 0.10°.

The first differential of the directional sensitivity curves (fig. 32) gives the rate of effective intensity change within a retinula cell as a point source moves through its visual field. The maximum rate of intensity reduction per degree is smaller in dark-adapted than in light-adapted cells as follows from the different slopes of the directional sensitivity curves. In both dark and light-adapted cells, however, the maximum rate of effective intensity change (40%/degree in light-adapted, and 20-25%/degree in dark-adapted cells) occurs at half the inter-ommatidia angle.

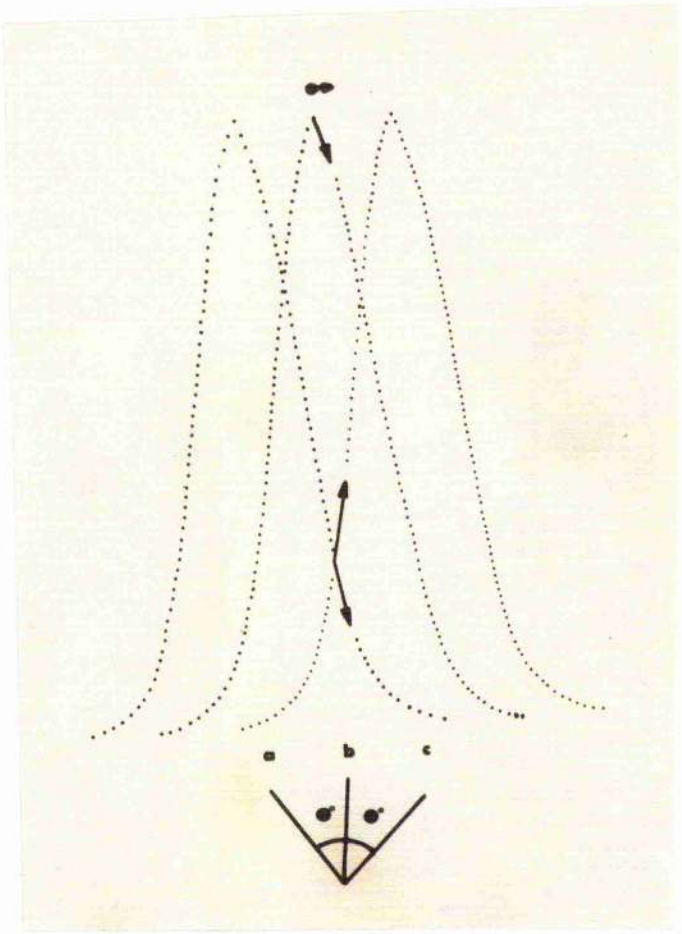


Fig. 31. The directional sensitivity of three cells (a,b and c) on neighbouring ommatidia is represented by the dotted curves. When a light source is moved from the axis of cell b the intensity perceived by cells a and b falls by different amounts, and the intensity perceived by c increases. These changes are indicated by the solid lines Θ° = the inter-ommatidial angle.

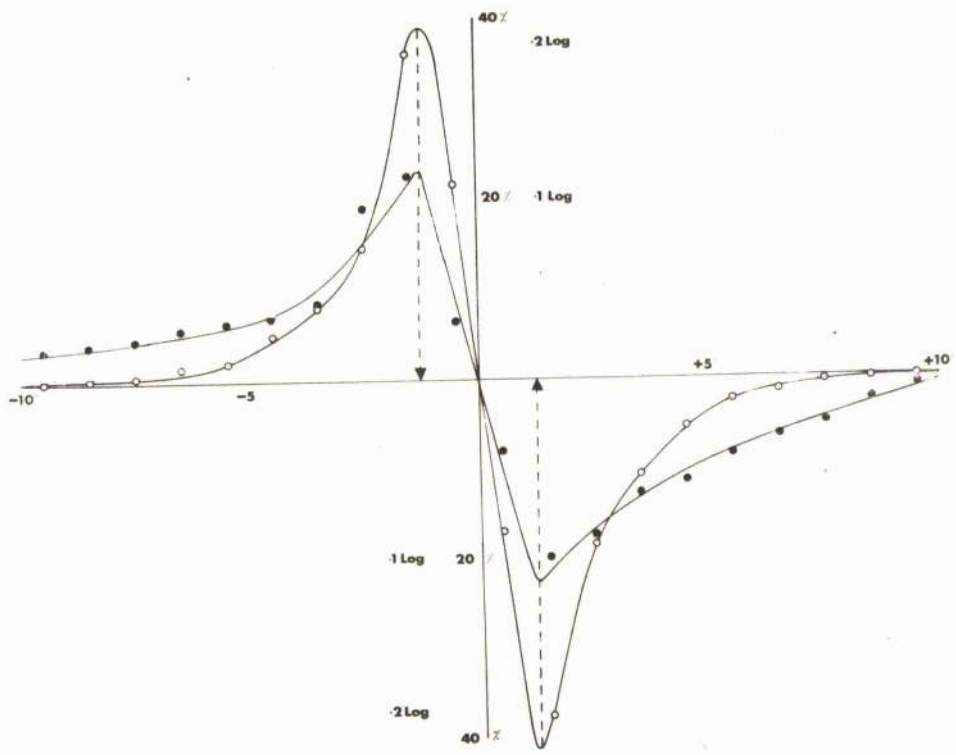


Fig. 32. The first differential of the directional sensitivity curves of dark-and light-adapted cells, showing the rate of effective intensity change in a retinula cell as a point source moves through its visual field.

Therefore, as a point source moves from the axis of one cell toward the axis of a second, the intensity perceived by the first is falling maximally just at the time when the intensity perceived by the second is increasing maximally and when the source is half-way between the two cells. Paired ommatidia can therefore, provide information of magnitude, velocity and direction of the movement of bright sources.

When a contrasting edge moves across the field of view of a retinula cell the situation is quite different. The maximum sensitivity to movement now lies when the edge is on the axis. At this position half of the field is dark, half illuminated and the edge is passing through the position at which the cell is maximally sensitive.

One of the most important consequences of the directional sensitivity of single cells and of the overlap of the visual fields of adjacent cells will be the influence these factors have on visual acuity. In the dark/light adaptation experiments reported above all other factors which might influence acuity, such as the resolving power of the dioptric apparatus, the inter-ommatidial angle and the nervous connections between ommatidia, remained constant while the angle of acceptance and the absolute sensitivity to light changed.

The influence of visual field overlap and dark adaptive increases in overlap on the contrast between adjacent ommatidia can be estimated approximately from the sensitivity distribution of single cells. In fig. 33 the contrast between ommatidia is plotted against the angle between them, using the results of sec. 3:2 and figure 17. Let us now

consider the situation where two point sources of equal intensity are situated on the axes of ommatidia a and c which are separated from each other by a third ommatidium b (see inset to figure 33). In the case where there is no overlap of the visual fields of adjacent ommatidia, then a and c would be stimulated maximally and b not at all. A situation of high contrast would then exist between ommatidia a and b or b and c. This would be the situation if Δ° was large relative to Δ p. In the case where the visual fields of neighbouring units do overlap ommatidium b will be stimulated by both lights, and this will result in a loss of contrast between adjacent ommatidia, and if there is a very large overlap source A will stimulate ommatidium c and conversely source B will stimulate ommatidium a, and this will result in further loss of contrast.

In fig 33 the intensity perceived by ommatidium b is plotted relative to the intensity perceived by a and c. The effective intensities in each are determined assuming that the directional sensitivity curves of the three ommatidia are equal and symmetrical, so that the intensity perceived by (a) is equal to the intensity perceived by (c), i.e. Ia = Ic.

In the calculation Ia and Ic are both represented as I ac. Ib is the sum of the intensity ommatidium b perceives from both sources.

The function $\frac{Iac - Ib}{Iac}$ represents the intensity contrast between ommatidia a or c and b (Reichardt 1962).

From the figure several things are seen. At the inter-ommatidial angle (2.4°) a light-adapted ommatidium b "sees" 60% of the light

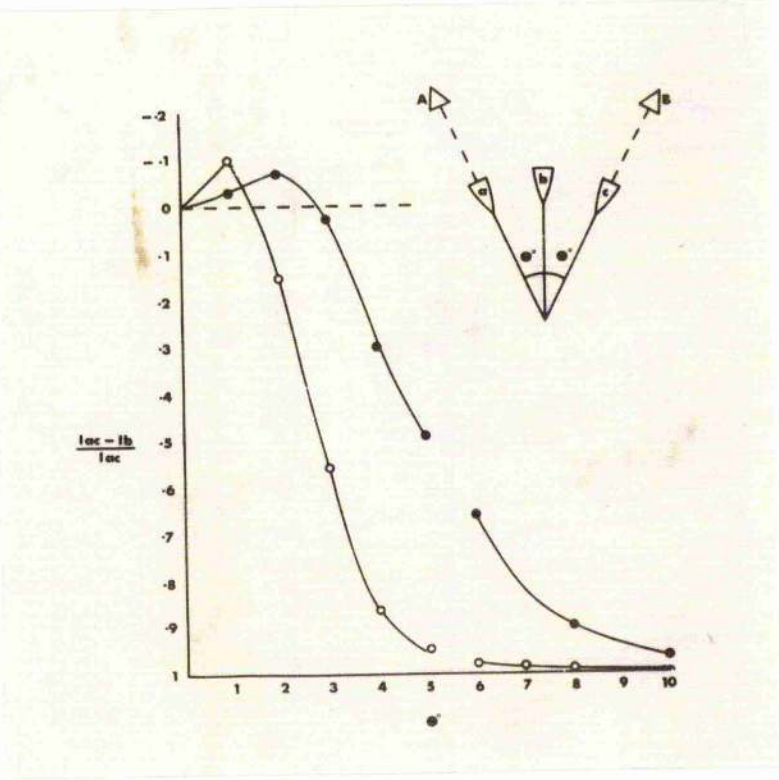


Fig. 33. Brightness contrast, defined as $\frac{Iac - Ib}{Iac}$ between neighbouring ommatidia as a function of the angle between the optical axes and the overlap of visual fields. On the ordinate 0 represents zero contrast where all ommatidia are stimulated equally. Negative values indicate the ommatidium b receives more light than either a or c and positive values vice versa.

Closed circles - dark-adapted cells

Open circles - light-adapted cells.

intensity incident at either a or c and there is a contrast of 0.4 between immediately adjacent ommatidia. With progressive dark-adaptation the proportion of light perceived by ommatidium b increases as its sensitivity to off-axis stimulation increases, until in the fully dark-adapted state contrast the adjacent ommatidia is reversed, i.e. ommatidium b now receives more light than either ommatidia a or c. In the figure zero contrast, i.e. when all ommatidia are stimulated equally, is represented by the horizontal dashed line. These considerations show that the overlap of the visual fields of adjacent ommatidia reduces contras between ommatidia and may even abolish or reverse it. Whether the loss of acuity which must result from overlap at the ommatidial level can be compensated for by a nervous integrating system, as is possible by lateral inhibition, has little experimental support in insect eyes.

In the preceeding paragraphs it has been assumed that the visual field of the whole ommatidium is the same as that of a single cell. Few experiments in this thesis have direct bearing on this point. In the study of the angular relationship between the axes of neighbouring visual cells (see 3:C), a close correspondence between the axis divergence of single cells and the inter-ommatidial angle was noted. This observation suggests a common physiological and anatomical axis. On the other hand, however, there are no recorded instances of two cells, giving good responses, sharing the same visual axis. It is possible that the advancing electrode did not successfully enter two cells within the same ommatidium or did so without appreciable change in the membrane potential

which would indicate a change of cells. The only satisfactory test of the hypothesis that all retinula cells in an ommatidium with a closed rhabdom have a common visual axis would be to record simultaneously the visual fields of two marked cells in a single ommatidium and to note differences, if any, in the position of their visual axes. The individual rhabdomeres in the losuct rhabdom are so closely apposed to one another, however, that one cannot imagine how there could be any differences between the visual field of a single cell and the visual field of the whole ommatidium, unless only the region adjacent to the cone is considered (see fig. 4 and page 16). It is probable that all the retinula cells in the closed rhabdom ommatidium have a common axis, and are depolarised equally by white light, although it is impossible to decide from the present experimental data whether this is in fact the case.

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